

From the Department of Women's and Children's Health  
Karolinska Institutet, Stockholm, Sweden

**LATE EFFECTS AND HEALTH-RELATED OUTCOMES  
AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL  
TRANSPLANTATION IN CHILDHOOD**

Mari Wilhelmsson



**Karolinska  
Institutet**

Stockholm 2018

Cover illustration by Mari Wilhelmsson.

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet.

Printed by Universitetsservice-AB

© Mari Wilhelmsson, 2018

ISBN 978-91-7676-944-7

# **LATE EFFECTS AND HEALTH-RELATED OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDHOOD**

**THESIS FOR DOCTORAL DEGREE (Ph.D.)**

Karolinska University Hospital, Astrid Lindgren Children's Hospital,  
Skandiasalen Q3:01, 1<sup>st</sup> floor

**Friday May 25<sup>th</sup>, 2018, 09.00 a.m.**

By

**Mari Wilhelmsson, MD**

*Principal Supervisor:*

Professor Kirsi Jahnukainen  
Karolinska Institutet  
Department of Women's and Children's Health  
Division of Pediatric Endocrinology

*Opponent:*

MD, PhD Dorine Bresters  
Leiden University  
Department of Paediatrics  
Division of Haematology and Oncology

*Co-supervisors:*

Professor Jacek Winiarski  
Karolinska Institutet  
Department of CLINTEC  
Division of Pediatrics

*Examination Board:*

Asst. professor Kristina Carlson  
Uppsala University  
Department of Medical Sciences  
Division of Haematology

Professor Henrik Hasle  
Aarhus University  
Department of Clinical Medicine  
Department of Paediatrics

Professor Bo Angelin  
Karolinska Institutet  
Department of Medicine  
Unit of Metabolism

MD, PhD Birgit Borgström  
Karolinska Institutet  
Department of CLINTEC  
Division of Pediatrics

Professor Klas Blomgren  
Karolinska Institutet  
Department of Women's and Children's Health  
Division of Pediatric Oncology



To my family for their love and support,  
and my father, in memoriam.



## ABSTRACT

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established treatment for many acquired or congenital disorders of the hematopoietic system. For some patients it may be the only curative option. Most children become long-term survivors and late toxicities are a major concern as their impact on health and quality of life can be serious. Therefore, a better understanding of the patterns of long-term toxicities and their risk factors is needed for more tailored treatment planning, follow-up programs and patient counseling. The general aim of the thesis was to study the spectrum of late toxicities in long-term survivors of pediatric allo-HSCT, to identify risk factors for adverse events and assess the additive toxicity associated with allo-HSCT in the treatment of childhood acute myeloid leukemia (AML).

In a retrospective case-note review, data was extracted from medical records of 204 allo-HSCT survivors with  $\geq 4$  years' follow-up after allo-HSCT. Special focus was placed on gonadal function and pubertal development in 96 female allo-HSCT survivors (Paper I) and in 102 male survivors (Paper II). The burden of late adverse events was analyzed for the whole cohort of long-term survivors (Paper III) and the impact of various conditioning regimens based on cyclophosphamide (Cy), busulphan (Bu), single fraction or fractionated total body irradiation (sTBI or fTBI) was evaluated. In order to assess the additive late toxicity associated with allo-HSCT in the treatment of childhood AML, questionnaire data derived from 95 Nordic childhood AML survivors treated with allo-HSCT was compared with corresponding data collected previously from 101 childhood AML survivors treated according to the common Nordic AML treatment protocols but without allo-HSCT; siblings of allo-HSCT survivors were used as a second control group (n=53) (Paper IV).

The burden of endocrine late effects was high after pediatric allo-HSCT; 38% had been treated with growth hormone, 38% had thyroxine substituted hypothyroidism, 50% had been treated with sex steroids, and 84% had at least one non-endocrine chronic health condition. TBI-based conditioning regimens were associated with the highest numbers of endocrine disorders, whereas the main risk factor for non-endocrine chronic conditions was chronic Graft-versus-Host Disease (Paper III). The risk of ovarian failure was high after both TBI- and Bu-based conditioning regimens and more than half (66%) of the female survivors needed hormone replacement therapy at their latest visit (Paper I). For male survivors, the recovery of spermatogenesis after allo-HSCT appeared more likely after chemotherapy-based conditioning regimens. Larger adult testicular volumes correlated with an active spermatogenesis suggesting that adult testicular volumes above 15mL may predict recovering spermatogenesis after allo-HSCT (Paper II). In the treatment of childhood AML, allo-HSCT was associated with significantly higher numbers of self-reported chronic conditions and health limitations, supporting the restriction of allo-HSCT to selected high-risk patients in first complete remission, whereas allo-HSCT without TBI after relapse may increase the risk of cardiovascular disorders (Paper IV). Several natural pregnancies were reported after allo-HSCT in childhood or adolescence (Papers III and IV). Our findings contribute to the expanding pool of knowledge on late complications after pediatric allo-HSCT.

## LIST OF SCIENTIFIC PAPERS

This thesis is based on the following publications. The papers in this thesis will be referred to by their Roman numerals.

- I. Vatanen A, **Wilhelmsson M**, Borgström B, Gustafsson B, Taskinen M, Saarinen-Pihkala U-M, Winiarski J, Jahnukainen K. Ovarian function after allogeneic hematopoietic stem cell transplantation in childhood and adolescence. *Eur J Endocrinol*. 2013 Dec 27;170(2):211-8.
- II. **Wilhelmsson M**, Vatanen A, Borgström B, Gustafsson B, Taskinen M, Saarinen-Pihkala UM, Winiarski J, Jahnukainen K. Adult testicular volume predicts spermatogenetic recovery after allogeneic HSCT in childhood and adolescence. *Pediatr Blood Cancer*. 2014 Jun;61(6):1094-100.
- III. **Wilhelmsson M**, Vatanen A, Borgström B, Gustafsson B, Taskinen M, Saarinen-Pihkala UM, Winiarski J, Jahnukainen K. Adverse health events and late mortality after pediatric allogeneic hematopoietic SCT—two decades of longitudinal follow-up. *Bone Marrow Transplantation* (2015) 50, 850–857.
- IV. **Wilhelmsson M**, Glosli H, Ifversen M, Abrahamsson J, Winiarski J, Jahnukainen K, Hasle H. Long-term health outcomes in survivors of childhood AML treated with allogeneic HSCT: A NOPHO –AML Study, *Manuscript*.



# CONTENTS

Background .....	3
1 Review of the literature .....	4
1.1 Allogeneic hematopoietic stem cell transplantation .....	4
1.1.1 Origin of allo-HSCT .....	4
1.1.2 Allo-HSCT indications .....	4
1.1.3 Hematopoietic stem cells .....	4
1.1.4 Donor .....	5
1.1.5 Conditioning regimens .....	6
1.1.6 Graft-versus-Host disease .....	7
1.1.7 Graft-versus-Leukemia effect .....	8
1.2 The treatment of childhood AML .....	9
1.3 Adverse events after allogeneic HSCT in childhood and adolescence .....	11
1.3.1 Acute complications .....	11
1.3.2 Late effects .....	12
1.3.3 Endocrine disorders, pubertal development and fertility .....	12
1.3.4 Bone growth and skeletal late effects .....	14
1.3.5 Cardiovascular and metabolic disorders .....	14
1.3.6 Pulmonary complications .....	14
1.3.7 Gastrointestinal disorders .....	15
1.3.8 Renal dysfunction .....	15
1.3.9 Ocular late effects .....	15
1.3.10 Dental late effects .....	15
1.3.11 Secondary malignancies .....	15
1.3.12 Neurocognitive deficits .....	16
1.3.13 Late effects after childhood AML .....	16
2 Aims of the study .....	19
3 Patients and methods .....	21
3.1 Long-term survivors of allogeneic HSCT (papers I-III) .....	21
3.1.1 Study population .....	21
3.1.1 Treatment characteristics .....	23
3.1.2 Methods .....	23
3.1.3 Statistical methods .....	24
3.1.4 Ethical considerations .....	24
3.2 Long-term survivors of childhood AML treated with allo-HSCT (paper IV) .....	25
3.2.1 Study design .....	25
3.2.2 Study population .....	25
3.2.3 Methods .....	25
3.2.4 Statistical analyses .....	26
3.2.5 Ethical considerations .....	26
4 Results .....	27

4.1.1	Ovarian failure and premature menopause (I).....	27
4.1.2	Recovery of spermatogenesis and testicular volumes (II) .....	29
4.1.3	Adverse events and late mortality (III).....	31
4.1.4	Additive toxicity associated with allo-HSCT in childhood AML treatment (IV) .....	34
5	Discussion.....	38
5.1.1	Ovarian failure and premature menopause after pediatric allo- HSCT (I) .....	38
5.1.2	Testicular function after pediatric allo-HSCT (II) .....	38
5.1.3	Adverse events and late mortality after pediatric allo-HSCT (III) .....	40
5.1.4	Long-term health-related outcomes after allo-HSCT for childhood AML (IV) .....	41
5.1.5	Strengths and limitations.....	42
5.1.6	Summary and conclusions .....	43
5.1.7	Future perspectives .....	44
6	Acknowledgements .....	46
7	References .....	49

## LIST OF ABBREVIATIONS

aGVHD	Acute Graft-versus-Host-Disease
AE	Adverse event
ALL	Acute lymphoblastic leukemia
Allo-HSCT	Allogeneic hematopoietic stem cell transplantation
AMH	Anti-Müllerian hormone
AML	Acute myeloid leukemia
APC	Antigen presenting cell
ATG	Anti-thymocyte globulin
BO	Bronchiolitis obliterans
Bu	Busulphan
CB	Cord blood
CTCAE	Common Terminology Criteria for Adverse Events
cGVHD	Chronic Graft-versus-Host disease
CRT	Cranial radiotherapy
CML	Chronic myeloid leukemia
Cy	Cyclophosphamide
FLT3/ITD	FLT3 internal tandem duplication
FSH	Follicle stimulating hormone
fTBI	Fractionated total body irradiation
G-CSF	Granulocyte colony-stimulating factor
GH	Growth hormone
GVL	Graft-versus-Leukemia effect
HLA	Human leukocyte antigen
HRT	Hormone replacement therapy
HSCs	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplantation
LH	Luteinizing hormone
MAC	Myeloablative conditioning regimen
MFD	Matched family donor
MHC	Major histocompatibility complex

MMUD	Mismatched unrelated donor
MRD	Minimal residual disease
MUD	Matched unrelated donor
NMA	Non-myeloablative conditioning regimen
NOPHO	The Nordic Association of Paediatric Hematology and Oncology
OR	Odds ratio
RIC	Reduced intensity conditioning regimen
SAA	Severe aplastic anemia
SCID	Severe combined immunodeficiency
sTBI	Single fraction total body irradiation
TBI	Total body irradiation
TNI	Total lymph nodal irradiation
TRM	Treatment-related mortality
TSH	Thyroid stimulating hormone

“One day, in retrospect, the years of struggle will  
strike you as the most beautiful.”  
— Sigmund Freud

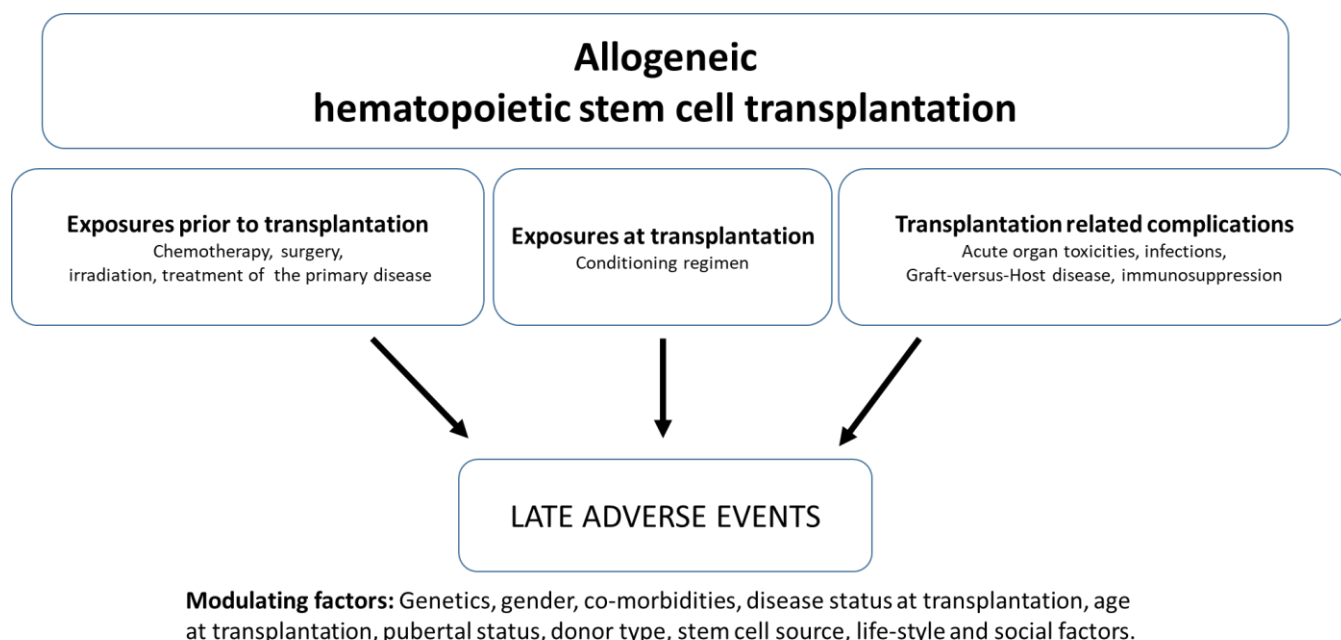


## BACKGROUND

This thesis explores the spectrum of late adverse events after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in childhood or adolescence. It also aims to identify risk factors for adverse events and reveal the additive toxicity associated with allo-HSCT in the treatment of childhood myeloid leukemia. Most children who receive allo-HSCT are expected to become long-term survivors and late effects research is therefore increasingly important.

There is often a long latency before the impact of changes in the present treatment protocols and conditioning regimens on long-term health effects can fully be appreciated. Once the late toxicities become apparent, treatment regimens may already be altered and the findings may not always be applicable to more currently treated patients. However, for the expanding population of long-term survivors even the late toxicities of previous regimens are highly relevant.

Recognition of late toxicities provides a basis for planning comprehensive follow-up guidelines and may also influence the future treatment regimens. The burden of late effects after allo-HSCT is influenced and modified by several factors. The exposures before, during and after transplantation all contribute to the spectrum and severity of late effects, schematically illustrated in Figure 1. More effective follow-up strategies with tailored screening and interventions can help modify and improve late outcomes and may ultimately help improve the health-related quality of life for survivors.



**Figure 1.** Factors that can be involved in the development of late adverse events after allogeneic HSCT.

# **1 REVIEW OF THE LITERATURE**

## **1.1 ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

### **1.1.1 Origin of allo-HSCT**

The potential of bone marrow cells was discovered in the 1950s when it was observed that intravenous injection of bone marrow cells to irradiated mice could re-establish their blood cell production (1). The research field developed rapidly and in 1957 came the first report describing allo-HSCT in humans leading to the procedure as we know it today (2). In an allogeneic transplantation the hematopoietic system of a patient is replaced or repopulated by an intravenous infusion of hematopoietic stem cells derived from a related or an unrelated donor, whereas in an autologous HSCT recipient's own stem cells are reinfused. Ever since the late 1970s allo-HSCT has been offered as a curative approach to an increasing number of patients with congenital or acquired diseases involving the hematopoietic system.

### **1.1.2 Allo-HSCT indications**

During the year 2012 almost 69 000 HSCTs were performed worldwide (3) and the same year allo-HSCT accounted for 42% of the pediatric stem cell transplantations in Europe (4). The largest disease indications were acute lymphoblastic leukemia (ALL) accounting for 26% and acute myeloid leukemia (AML) accounting for 14% of all pediatric allo-HSCTs in Europe. The largest non-malignant disease indications included primary immunodeficiency (16%), bone marrow failure (12%) and thalassemia (8%) (4). Other allo-HSCT indications include myelodysplastic syndrome (MDS), juvenile monomyelocytic leukemia (JMML), chronic myeloid leukemia (CML) and non-Hodgkin lymphoma (NHL), severe aplastic anemia (SAA), severe combined immunodeficiency (SCID), and other severe T-cell and granulocyte disorders, hemophagocytic histiolymplocytosis, Diamond Blackfan anemia, sickle cell anemia, Fanconi anemia, and some inborn errors of metabolism (such as Morbus Hurler). New disease indications are constantly being explored and pediatric stem cell transplantations for non-malignant diseases have greatly increased during the past decades (5). At least one third of the pediatric allo-HSCTs have a non-malignant disease indication.

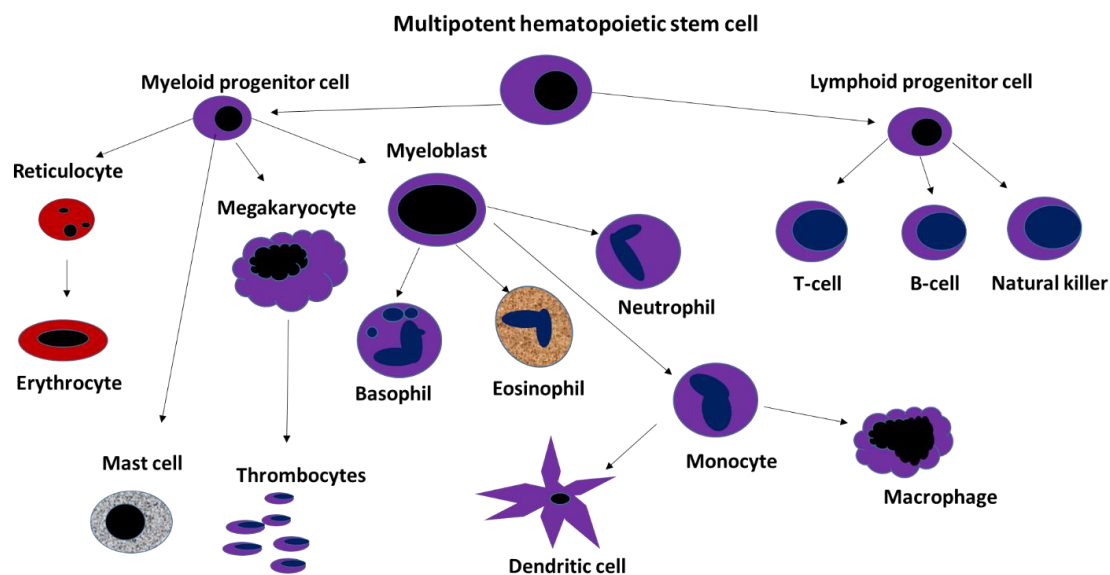
### **1.1.3 Hematopoietic stem cells**

In allo-HSCT, donor hematopoietic stem cells (HSCs) are given in order to replace or repopulate the bone marrow. Stem cells can divide and have the capacity to differentiate into other cell lineages (Figure 2). CD34, a cell surface glycoprotein, is the most commonly used surrogate marker for identifying hematopoietic stem cells (6) and the total given number of CD34+ cells is considered to be prognostic in stem cell transplantation (7, 8).



The three primary sources of HSCs include:

- 1) Bone marrow – stem cells are obtained from the bone marrow through bone marrow aspiration.
- 2) Peripheral blood stem cells (PBSCs) – granulocyte colony stimulating factor (G-CSF) is given in order to mobilize PBSCs from bone marrow into peripheral blood and PBSCs are collected from peripheral blood with apheresis.
- 3) Umbilical cord blood (CB) – stem cells are collected from blood vessels of the placenta after childbirth and cryopreserved for later use.



**Figure 2.** The Hematopoietic System.

#### 1.1.4 Donor

The availability of a suitable donor can limit the use of allo-HSCT. The choice of a donor is guided by tissue compatibility or histocompatibility antigens located on chromosome 6. A well-matched donor matches for at least 9 of the 10 alleles of the human leukocyte antigen (HLA) system that encodes the major histocompatibility complex (MHC) proteins (9, 10). An ideal HLA-match matches 10/10 alleles and can be found for approximately half of the patients with Western European ancestry; for an additional 20–30% a match for 9/10 alleles is usually available (11).

An HLA-matched sibling, if available, is often chosen. The chance of an individual sibling being an HLA-match is 25%. If a matched sibling donor is lacking, or not considered to be the most ideal, international unrelated donor registries may be searched for a matched unrelated donor (MUD). If a matched donor cannot be found within a satisfactory timeframe, a mismatched (haploidentical) related or unrelated donor can be considered. Modern graft processing technologies have enabled the safe use of haploidentical donors (12), and especially parental donors are often readily available.

### 1.1.5 Conditioning regimens

Before the donor stem cells can be given the recipient needs to be prepared with a conditioning regimen. The purpose of conditioning regimens is to suppress the host's bone marrow in order to allow engraftment of the donor cells. The choice of conditioning is mainly based on the disease indication. In malignant disease, a myeloablative conditioning (MAC) that eradicates the host's hematopoiesis without allowing spontaneous recovery is usually chosen for eradicating minimal residual disease (MRD).

#### *Total body irradiation*

Total body irradiation (TBI) eradicates MRD and is immunosuppressive. The advantages of a TBI-based conditioning include its independence from drug absorption, metabolism or transport across the blood–brain barrier. TBI is usually combined with a chemotherapeutic agent, most commonly with cyclophosphamide (Cy), etoposide or cytarabine (ARA-C). TBI has been an important part in the preparative regimens for malignant disease for decades. Up to the 1980s conditioning regimens with TBI and/or Cy were preferred. In the earlier era, TBI was often given as a single fraction TBI (sTBI) of 10–12 Gy. Due to its high toxicity sTBI has been replaced by less toxic fractionated TBI (fTBI). fTBI is also often given as 10–12 Gy but divided into several fractions, usually of 2–4 Gy, over a period of two or three subsequent days. In the treatment of childhood AML, TBI has been replaced by Bu whereas in ALL, it is yet unproven whether TBI can be replaced by chemotherapy-based conditioning regimens without compromising survival (13). The results of an ongoing multinational randomized controlled trial, the ALL SCTped 2012 FORUM study, aimed at addressing this question are yet to be published.

#### *Chemotherapy-based myeloablative conditioning regimens*

Bu has been offered as the myeloablative alternative to TBI since the early 1980s (14). It is usually given in combination with Cy (14). The oral administration of Bu has a highly varying bioavailability but by using intravenous administration with a pharmacokinetic directed dosing the systemic exposure can be more easily controlled. The systemic exposure of Bu has a strong correlation to the regimen-related severe adverse events (15).

Myeloablative alternatives to Bu with less toxic profiles have been employed especially in non-malignant disease. Alkylating agents such as treosulfan, trophosphamide, melphalan and thiotepa and an antimetabolite fludarabine have been used in various combinations. For patients with SAA, Cy-based conditioning regimens are usually used, sometimes in combination with fludarabine and/or a low dose of TBI.

### *Reduced intensity conditioning regimens*

If the patient history suggests that the patient may not tolerate full myeloablative conditioning, for example in case of previous myeloablative therapy or severe infections, a RIC regimen may be employed. In RIC regimens alkylating agents or TBI are generally reduced by one third (or more), making these regimens less toxic. Fludarabine is often the main agent used in combination with intermediate doses of alkylating agents like Bu. In malignant disease RIC regimens depend more on the graft-versus-leukemia effect for preventing relapse. The experience from RIC in the pediatric population is limited, but it has been well tolerated in recent clinical trials in the treatment of childhood AML in combination with immunotherapy (16). The reported survival rates after RIC in the treatment of pediatric AML have been comparable with MAC (17).

### *Non-myeloablative conditioning regimens*

Non-myeloablative regimens are seldom used in children (except for SAA). The non-myeloablative (NMA) regimens often contain TBI in low doses (<2 Gy) combined with fludarabine or Cy (18). NMA suppresses the immune system to enable engraftment but it does not eradicate host hematopoiesis, thus allowing the hematopoiesis to recover quickly, and at engraftment mixed chimerism is expected (19).

#### **1.1.6 Graft-versus-Host disease**

In GVHD, T-cells derived from the donor interact with activated host antigen-presenting cells (APCs); the recognition of the presented host peptides as foreign leads to attack against host cells and tissue damage (20). GVHD was the major obstacle in the early days of allo-HSCT and it is still a major cause of mortality and morbidity, although reports indicate that the proportion of grade III–IV acute GVHD declined by 20% between 1999 and 2012 (21). Many factors are likely to have contributed to the decrease, such as improved genomic donor/recipient matching, less frequent use of TBI and T-cell depletion techniques including the increased use of anti-thymoglobuline (ATG). Still, about 35–70% of allo-HSCT recipients develop acute GVHD and 20–50% develop chronic GVHD; the rates are influenced by type of transplant, patient characteristics, and GVHD prophylaxis regimen (21).

Acute GVHD (aGVHD) has traditionally been defined as Graft-versus-Host occurring within the first 100 days after transplantation. The cytokine storm of aGVHD results in direct tissue damage, generally restricted to the skin, gastrointestinal tract and liver.

Chronic GVHD (cGVHD) usually occurs with a more delayed presentation 100 days or later after allo-HSCT, involving a broader range of organs and having features that resemble autoimmune disorders (20). It was traditionally staged as limited or extensive according to the Seattle criteria (22), but the staging was revised by the National Institutes of Health (NIH) consensus meeting and includes now three grades: limited, moderate and severe (23). Studies indicate that different mechanisms are involved in the development of aGVHD and cGVHD

(24) and they can overlap and be present simultaneously (25). NIH classification has divided aGVHD into classical and persistent (late onset) aGVHD (23).

The risk of GVHD is influenced by the source of HSCs, lower risk of cGVHD having been observed with cord blood source and higher risk with PBC source. The risk of cGVHD increases with increasing donor mismatch when un-manipulated graft is used and ranges from 6% (26) to as high as 65%. T-cell depletion techniques at transplantation can reduce alloreactivity of GVHD, but may at the same time diminish the important Graft-versus-Leukemia effect associated with allo-HSCT (27).

Corticosteroids are the first-line treatment for both aGVHD and cGVHD but optimal in only about half of patients (21). For aGVHD prophylaxis after MAC allo-HSCT, a majority of the European transplant centers use cyclosporine combined with a shorter course of methotrexate (MTX) (28). Many different immunosuppressive approaches have been used in the management of cGVHD (21) including MTX, calcineurin inhibitors, mycophenolate mofetil, pentostatin, sirolimus, daclizumab, anti-tumor necrosis factor, tyrosine kinase inhibitors and extracorporeal photopheresis (ECP). In ECP leukocytes are collected from peripheral blood, photosensitized and re-infused after exposure to ultraviolet irradiation by using apheresis equipment (29).

#### **1.1.7 Graft-versus-Leukemia effect**

In the treatment of malignant disease GVHD cannot only be regarded as a complication. The strong immunological force against tumor cells, Graft-versus-Leukemia effect (GVL), was already observed by Thomas et al. in 1977 (30) when leukemia patients with GVHD showed lower relapse rates. GVL is partly mediated through T cells that detect MHC-bound target peptides on leukemic cells. Clinical observations indicate that GVHD and GVL often occur in the same patient and the underlying mechanisms are similar if not identical (27), and the prevention of GVHD without interfering with GVL is therefore a major challenge.

In autologous HSCTs this important antileukemic phenomenon is lacking, and in the treatment of leukemia allo-HSCT can be considered superior to autologous HSCT. The benefit of using auto-SCT in the first complete remission (CR1) of pediatric AML seems to be marginal compared to chemotherapy only (31). At present, autologous HSCT serves mainly as autologous stem cell support after high-dose chemotherapy regimens in the treatment of malignant tumors.

## 1.2 THE TREATMENT OF CHILDHOOD AML

Allo-HSCT has played an important role in the treatment of childhood AML ever since the first publication came in the late 1970s showing that allo-HSCT could cure patients with AML (30). International collaboration has been the key to progress in treating this heterogeneous and rare childhood cancer with an incidence rate of 7 per million children per year. The current survival rates are around 70% (32).

### *Induction and consolidation therapy of childhood AML*

The treatment of AML is very intensive and treatment-related mortality is relatively high, around 10% (33, 34). AML treatment is based on anthracyclines and anti-metabolites. Cytarabine is combined with anthracycline and the standard induction therapy comprises three days of anthracyclines and 7–10 days of cytarabine. With these regimens > 85% of the pediatric patients enter complete remission (CR) (32). In most groups AML therapy consists of five courses of chemotherapy in total, with one or 2 courses of induction and three consolidation courses. CNS directed intrathecal therapy is routine but the majority, if not all pediatric study groups, have stopped using cranial radiotherapy (CRT). The Nordic Society for Paediatric Haematology and Oncology (NOPHO) protocols for AML have not included CRT. Instead, methotrexate has been used for CNS prophylaxis, and the treatment of CNS leukemia in the NOPHO-AML 2004 protocol included intrathecal triple therapy with cytarabine, methotrexate and prednisone. The doses of cytarabine, etoposide and cumulative doses of anthracyclines in the NOPHO-AML protocols are shown in Table 1.

Protocol	NOPHO-AML -84	NOPHO-AML -88	NOPHO-AML -93	NOPHO-AML 2004	NOPHO-AML 2012		
					SR without inv(16)	SR with inv(16)	High risk
Cytarabine (g/m <sup>2</sup> )	50.4	50.1	*49.6 ** 61.3 1600	49.3	36.8 or 45.4	30.8 or 39.4	8.8 or 17.4
Etoposide (mg/m <sup>2</sup> )	0	1600		1200	1200 or 750	1200 or 750	1200 or 750
Cumulative dose of anthracyclines (mg/m <sup>2</sup> )	225	450	*300 **375	480	180 or 360	180 or 360	180 or 360
Fludarabine (mg/m <sup>2</sup> )	-	-	-	-	150 or 300	150 or 300	0 or 150

**Table 1.** Cytarabine, etoposide and anthracycline doses in the NOPHO-AML-84/88/93/2004/2012 protocols. SR indicates standard risk, \*) good responders, \*\*) poor responders.

### *Treatment of relapsed AML*

Relapse rates vary between 21-40% in the different study groups making relapse a major cause of treatment failure (35). The NOPHO-AML 2004 protocol had a relapse rate of 30%. Currently, relapsed AML is treated with intensive re-induction with one or two courses followed by allo-HSCT once in CR2 or aplasia. No predefined strategy existed for relapse treatment during the NOPHO-84, -88 and -93 protocols.

### *The role of allo-HSCT in the treatment of childhood AML*

Most study groups advocate allo-HSCT after relapse but the role and timing of allo-HSCT has been controversial (36). While allo-HSCT in CR1 associates with an increased event-free survival, it seems to have only minimal effect on the overall survival (36). It has been estimated that ten AML patients would have to be transplanted in CR1 in order to prevent one relapse (37) and a significant proportion of patients can be cured after relapse (38). The proportion of pediatric AML patients who proceed to transplant varies greatly between the large childhood AML trials with figures ranging from 2 to 29% (35). Although no international consensus exists on timing of allo-HSCT there is some agreement on common markers for high-risk (HR) AML and risk-group stratification followed by risk-based treatment. Stratification to HR is determined by cytogenetics, residual disease (no remission after second induction) or relapse. Patients with HR AML, like patients with the FLT3-internal tandem duplication (ITD) mutation that associates with a poor prognosis, are likely to benefit from an allo-HSCT in CR1 (39). The challenge is to accurately identify all those patients who benefit from an early allo-HSCT in CR1 and who could thereby be spared from relapse.

### *Allo-HSCT in the NOPHO-AML protocols*

The indications for HSCT in CR1 in the NOPHO-AML protocols are listed in Table 2 (37, 38). The recommended conditioning regimen in the NOPHO-AML 2012 protocol is a myeloablative combination of Bu/Cy/melphalan, while for selected patients with previous severe organ toxicity a less toxic RIC regimen can be considered (40).

Protocol	Allo-HSCT indications in CR1	Donor	Allo-HSCT in CR1
<b>NOPHO-AML -84</b>	All patients with an available matched family donor	MFD	16%
<b>NOPHO-AML -88</b>	All patients with an available matched family donor	MFD	21%
<b>NOPHO-AML -93</b>	Only high-risk patients with matched family donor	MFD	27%
<b>NOPHO-AML 2004</b>	>15% blasts on day 15 or no remission after second induction or MLL dearrangements other than (t9;11)(p21;q23). From 2009: Poor response to induction only.	MFD or MUD	13%
<b>NOPHO-AML 2012</b>	Patients with a poor response after 2 induction courses, patients with FLT3-ITD without NPM1 mutation	MFD or MUD	Estimated 7-10% eligible

**Table 2.** The indications for allo-HSCT in CR1 in the NOPHO-AML-84/88/93/2004/2012 protocols and the proportion of patients transplanted in CR1. CR1 indicates first complete remission; MFD, matched family donor; MUD matched unrelated donor.

### **1.3 ADVERSE EVENTS AFTER ALLOGENEIC HSCT IN CHILDHOOD AND ADOLESCENCE**

During the past decades great improvements have been made in supportive care, donor selection and the expanding experience on acute complications, with a subsequent reduction in transplant related deaths as well as an increase in long-term survival (41). However, the late treatment-related morbidity and mortality associated with allo-HSCT is still considerable and mortality rates may be twice as high when compared with the general population (42). Almost all the survivors of pediatric allo-HSCT will experience at least one late effect (43). While the endocrine late effects are most common, any organ may be affected. The type of conditioning regimen, age at transplantation as well as the presence of cGVHD have a major impact on the burden and spectrum of late effects (44-46).

#### **1.3.1 Acute complications**

##### *Acute injuries and infections*

The conditioning regimen can induce acute injuries and is usually behind liver injuries leading to sinusoidal obstruction syndrome, or Hepatic Veno-Occlusive Disease, a potentially fatal complication that usually occurs during the first 30 days after HSCT (47). Thrombotic microangiopathy is another complication deriving from endothelial injury with a high mortality rate of 50–60% (48). Posterior reversible encephalopathy syndrome (PRES) may also be related to vascular injury, and other neurological treatment-related complications are not uncommon. Hemorrhagic cystitis, characterized by hemorrhagic inflammation in urinary tract mucosa, is most likely caused by the toxicity of chemotherapy and irradiation at early presentation, whereas multiple causes including viral infections can be involved in late onset (49). Oral mucositis is a highly common complication affecting 47 (15) to 75% (50) of the patients after transplantation and increases the risk for infections. During the period of pancytopenia following transplantation, the risk of invasive fungal infections, opportunistic infections, bacteremia and viral reactivation is high.

##### *Graft failure*

In approximately 5% of the allo-HSCTs a graft failure can occur, which is associated with a reduced 5-year survival in malignant disease (51). The likelihood can be even higher in special cases, for example when mismatch is present or when RIC regimens are used. A chimerism analysis can be used to assess engraftment and graft failure early. Full chimerism is achieved earlier after MAC than RIC regimens (14) and subsequently graft rejection is more common after RIC regimens (52). Many factors other than conditioning intensity may be involved in the occurrence of graft failure, including HLA match, immunosuppression regimen, cell dose, drug toxicities and viral infections (53).

## *Acute Graft-versus-Host Disease*

Approximately one third or up to a half of the patients develop aGVHD which is usually limited to skin, gastrointestinal tract and liver (54). Based on the severity and number of organs involved it is staged and graded into four grades, 0–IV, where grades III and IV are associated with poor outcomes (54).

### **1.3.2 Late effects**

#### **1.3.2.1 Chronic Graft-versus-Host Disease**

cGVHD is an allo-HSCT specific complication and is associated with significant late morbidity affecting 20–50% of the allo-HSCT recipients (21). The incidence is lower in children than in adults and lower in matched sibling transplants than in MUD or mismatched grafts. cGVHD is considered one of the major barriers for achieving a high quality of life, and the resolution of cGVHD may significantly increase the health-related quality of life.

#### **1.3.3 Endocrine disorders, pubertal development and fertility**

Endocrinopathies and impaired growth are the most common long-term side effects affecting 60% of the pediatric allo-HSCT survivors (43, 55-57), with transplantation at a young age as well as TBI-based conditioning regimens identified as major risk factors (58). The most frequently affected organs include the thyroid gland, the gonads and the pituitary.

##### *Hypothyroidism*

Hypothyroidism may occur late. Due to the increasing cumulative incidence over time continued annual screening for hypothyroidism is recommended for 10 years after Bu-based conditioning and for 30 years after TBI (59). Rates of 30–60% have been reported in long-term survivors after TBI or Bu (60). The risk for hypothyroidism is highest after TBI while significantly lower rates have been reported after Cy-based conditioning regimens compared to TBI or Bu.

##### *Gonadal failure in female survivors*

Both TBI and Bu are highly gonadotoxic and primary gonadal failure is frequently reported in female survivors after allo-HSCT (61-63) and is influenced by the timing of allo-HSCT. Prepubertal girls with a larger ovarian reserve may have a better chance of spontaneous recovery even after TBI and/or high doses of alkylating agents. Many girls require medical induction of pubertal development and hormone replacement therapy (HRT) for regular menstruations and the risk of premature menopause is high (64).

Elevated levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) can indicate gonadal failure. Lower levels of Anti-Müllerian hormone (AMH) can indicate a diminished oocyte reserve and help in the prediction of menopause. In addition, an ultrasound can be used for measuring ovarian volume and Antral Follicular Counts (65). Premature



menopause with low estrogen levels can lead to osteoporosis, increase the risk of cardiovascular disorders and impair sexual and psycho-social well-being (66).

#### *Gonadal failure in male survivors*

The Leydig cells that are responsible for producing testosterone are relatively resistant to the effects of conditioning regimens and in most cases testosterone levels remain normal. Leydig cells can often retain normal function when radiation dosage to the testis is less than 20 Gy whereas direct testicular radiation above 20 Gy can cause permanent Leydig cell damage (67). In non-irradiated male survivors, pubertal development may be normal with the exception of testicular volumes (64, 68). A significant proportion of the survivors may develop Leydig cell insufficiency requiring testosterone treatment after cytotoxic drugs and irradiation (69). A study including 206 male survivors of pediatric allo-HSCT showed low testosterone levels ( $<2\mu\text{g/mL}$ ) in 18% of the male survivors and an additional 5% needed treatment with testosterone (63).

#### *Fertility*

One of the major concerns of allo-HSCT survivors is infertility. Limited data exist on fertility rates after allo-HSCT and the previously reported figures are very low. A large EBMT survey reported fertility rates below 1% for allo-HSCT survivors transplanted at all ages (70). However, some survivors may retain fertility even after myeloablative conditioning regimens (71). Very few reports have assessed involuntary childlessness among allo-HSCT survivors; however, pregnancies after allo-HSCT are regarded as rare events.

Pregnancies in female survivors of allo-HSCT can be regarded as high risk with previous studies showing an increased risk of premature delivery and cesarean section (70, 71). Irradiation to the pelvic area can cause direct damage to the myometrium and the endometrium of the uterus as well as to the uterine vasculature (72).

Male survivors have a very high risk of developing oligospermia or azoospermia after myeloablative conditioning regimens (63). In a cohort of 217 male HSCT survivors only one third (27%) had spermatozoa after a median follow-up time of 4.5 years (73). The sperm-producing germinal epithelium of the testes and Sertoli cells are highly sensitive to irradiation and already lower doses of 0.2 Gy can cause testicular injury reflected by increased levels of FSH (74), and irreversible azoospermia can be caused with doses above 4 Gy (64). Higher cumulative doses of alkylating agents can also seriously impact the spermatogenesis and cause azoospermia. Survivors with no exposure to cytotoxic agents prior to transplant may have a higher chance of recovering their spermatogenesis after allo-HSCT provided that no irradiation is given (75). However, persistent cGVHD may negatively impact recovering spermatogenesis and increase the risk of azoospermia (73, 76).

Based on current knowledge, the offspring of cancer survivors and allo-HSCT survivors have no increased risk of non-hereditary cancer or congenital malformations (77, 78).

### **1.3.4 Bone growth and skeletal late effects**

Growth is compromised in many survivors, and greater height deficits have been associated with younger age at time of allo-HSCT. Growth hormone (GH) deficiency is more likely after TBI-based conditioning regimens and is especially common in patients who have received CRT prior to allo-HSCT (79). Both TBI and GH deficiency associate with deficits in height and in the musculoskeletal system (80). While chemotherapy alone does not usually impair the hypothalamus-pituitary-gland-axis or cause GH deficiency, chemotherapy and irradiation can cause peripheral lesions in epiphyseal growth plates, cartilage and bones (81). Gonadal failure with delayed puberty, hypothyroidism and corticosteroid treatment can all contribute to impaired physical growth. High corticosteroid doses for long periods given as part of the leukemia treatment or as first line treatment of cGVHD, can cause osteoporosis and osteonecrosis.

Many survivors of allo-HSCT seem to benefit from growth hormone (GH) treatment with improved final heights (82). The safety of using of GH treatment in patients previously treated for malignant disease has been debated. A recent report showed no increase in the risk of cancer mortality in the GH-treated population but a trend towards increased cancer mortality was seen with increasing GH doses among patients previously treated for malignant disease (83).

### **1.3.5 Cardiovascular and metabolic disorders**

Compared to the general population, allo-HSCT survivors have an increased risk of cardiovascular disease. The risk of premature cardiovascular death has been 2.3- to 3.6-fold in previous reports (42, 84). Multifactorial causes may precede premature cardiovascular aging (85). Allo-HSCT survivors are at risk of developing insulin resistance and metabolic syndrome. In two large studies including both pediatric and adult HSCT survivors, the prevalence of diabetes was 14–17%, dyslipidemias and hypertension were detected in 44% and 28–36%, respectively (46, 86). The use of TBI has been associated with metabolic syndrome (87). While obesity is relatively uncommon after HSCT, a reduction in muscle and an increase in fat mass percentage can be present (88). Hypothyroidism, which is very prevalent among HSCT survivors, associates with unfavorable lipid profiles (85).

Irradiation can cause changes in arterial intima and induce premature cardiovascular aging (89). Higher cumulative doses of anthracyclines (exposures of 250 mg/m<sup>2</sup> or more) and cardiac exposure to irradiation increase the risk of cardiac disease in childhood cancer survivors (90). Comorbidities and treatments received prior to allo-HSCT have great impact and the risk of late congestive heart failure after allo-HSCT may primarily be determined by the anthracycline exposures prior to HSCT (90).

### **1.3.6 Pulmonary complications**

Pulmonary failure is a major cause of non-relapse late deaths after allo-HSCT; compared with the general population, allo-HSCT survivors' risk of death due to pulmonary

dysfunction can be increased by as much as a 15-fold (42). Bronchiolitis obliterans (BO) is the pulmonary manifestation of cGVHD characterized by irreversible small airway obstruction and once it has developed it has a poor prognosis (91). The occurrence of late-onset non-infectious pulmonary complications with BO, interstitial pneumonia and bronchiolitis obliterans organizing pneumonia (BOOP) may be lower after RIC regimens while TBI and Bu-based regimens can have direct pulmonary toxicity (92).

### **1.3.7 Gastrointestinal disorders**

Allo-HSCT survivors have a substantially higher risk for hospitalization for liver diseases (93). In the earlier era, before screening for hepatitis C or B, viral hepatitis was a relatively frequent complication associated with frequent blood transfusions. The hepatic iron overload that is prevalent after allo-HSCT usually dissolves spontaneously over the years but it can cause liver dysfunction and may be involved in the development of many extra-hepatic complications (94). Problems of the gastrointestinal tract are often closely related to the presence of cGVHD. cGVHD may present with a non-viral hepatitis, chronic diarrheas and malnutrition, and some survivors may develop strictures in the gastrointestinal tract requiring surgical dilation.

### **1.3.8 Renal dysfunction**

Acute or chronic renal dysfunction after allo-HSCT can be caused by nephrotoxicity from calcineurin inhibitors used for GVHD prophylaxis, antibiotics used for sepsis, tubular necrosis caused by ischemia or septicemia (95). Membranous nephropathy has been recognized as a HSCT-related cause of glomerular damage (95). cGVHD does not usually present itself in the urinary tract although microangiopathy has been proposed to be a renal manifestation of cGVHD. CMV and EBV reactivations are involved in late-onset hemorrhagic cystitis.

### **1.3.9 Ocular late effects**

Cataract, a highly common side effect affecting 30–80%, is closely related to irradiation, and many TBI recipients develop opacities within the first years after allo-HSCT (94) often requiring a cataract operation. cGVHD can present with dryness of eyes, keratoconjunctivitis sicca and corneal ulcerations.

### **1.3.10 Dental late effects**

Irradiation as part of the conditioning regimen can cause underdevelopment of the mandible, mandible joint and the teeth and the enamel, the shape of the teeth and roots may be defected (94). Oral manifestations of cGVHD include mucosal dryness and atrophy, and chronic stomatitis may decrease the quality of life.

### **1.3.11 Secondary malignancies**

Cumulative incidence rates of secondary malignancies after HSCT vary between 3.5 and 7% at ten years, and a rate of 12.8% at 15 years has been reported (42, 96). No plateau has been seen after 20 years of follow-up. The more frequently reported second solid tumors include

breast cancer, skin cancer and thyroid cancer (88). Irradiation and conditioning regimens including TBI are the major risk factor for developing secondary malignancies.

### **1.3.12 Neurocognitive deficits**

Frequent neurocognitive deficits have been reported after allo-HSCT and conditioning with TBI is an important risk factor. Especially in the youngest recipients of HSCT (age <3 years) a high percentage (78–85%) of long-term neurocognitive complications has been reported, with TBI and younger age at HSCT identified as risk factors (58, 97). During the earlier era, CRT was widely used in ALL with doses of 18–24 Gy for CNS leukemia prophylaxis, although effective; it has associated with serious neurotoxic side effects and neurocognitive dysfunction (98).

Survivors of pediatric allo-HSCT may also experience deficits in social skills when compared to sibling controls (99). Depression, anxiety, and psychological dysfunction are also frequently reported after HSCT, and among adult survivors of HSCT an increase was found in deaths due to suicide or accidents when compared with general population (100).

### **1.3.13 Late effects after childhood AML**

Some studies have been published comparing late toxicities after allo-HSCT for childhood AML treated with and without HSCT. The main results from these previous studies (101–107) are listed in Table 3.

In general, more favorable outcomes have been reported for survivors of childhood AML treated without HSCT. However, in a study that included 272 5-year survivors of childhood AML, the AML survivors had a significantly higher prevalence of a chronic health conditions (grades 3 or 4) when compared to their sibling controls (16% vs. 6%), and after 20 years from diagnosis the AML survivors had a cumulative incidence of 1.7% for secondary malignancies and 5% for cardiac events (108). The childhood AML survivors treated according to the NOPHO-AML protocols without HSCT or relapse have generally reported good health outcomes with normal pubertal development, and fertility has been comparable with their sibling controls (109–111). Although the conventionally treated AML survivors did not report more cardiovascular symptoms compared to healthy controls, a significant reduction of left ventricular function was seen that associated with increasing cumulative doses of doxorubicin (112). An increased risk of cardiovascular complications is correlated with increasing cumulative doses of anthracyclines and exposure to irradiation can further increase the risk (90). For relapsed AML survivors a higher 10-year cumulative incidence of cardiotoxicity has been reported when compared to AML survivors without previous relapse; 35% vs. 11%, respectively (101).

Number of patients	Follow-up, years	Therapy	Main findings	Reference
218	≥5 y Median 9 (range 5–14)	95 Chemo only 86 HSCT: 30 TBI	After HSCT in CR1: cardiotoxicity 8% vs. 14% after Chemo only (NS). No difference in self-reported Quality of Life.	Barlogis et al, 2015.
180	≥5 y Median 20 (range 9–31)	100 Chemo only 26 Auto-HSCT 54 Allo-HSCT: 25 (46%) TBI 29 (54%) no TBI	After HSCT: more chronic health conditions, grades 1-4 (76% vs. 44%), any grade 3 or 4 (33% vs. 16%). After allo-HSCT lower physical mean summary scores. Overall HRQL scores were similar between the groups.	Schultz et al, 2014.
21	≥5 y Median 20 (range 9–31)	12 Chemo only 6 allo-HSCT, TBI 3 auto-HSCT, TBI	After HSCT: more pituitary deficiencies and metabolic syndrome (18% vs. 5%), hypothyroidism (50 % vs. 0%), and dyslipidemia (63 % vs. 7%) compared to chemo only.	Blijdorp et al, 2013.
171	≥2 y	131 Chemo only 40 HSCT: 40 BuCy	After HSCT 73% had one or more late sequela (cardiomyopathy, liver dysfunction, skeletal anomalies, HRT) vs. 32% after chemo only.	Klusmann et al, 2012.
77	≥10 year Median >15 years (range 11–38)	44 Chemo only 18 Chemo+RT 15 HSCT: 15 TBI Cy	After HSCT: More affected weight and height z-scores, more growth hormone deficiency (27%), hypothyroidism (20%), hypogonadism (53%), infertility (100%), and cataracts (60%). Cardiovascular late effects were comparable between the groups (HSCT 7% vs. chemo 9%). More neurocognitive problems after Chemo+RT and allo-HSCT.	Leung et al, 2000.
52	≥1 year Chemo ± RT: mean 7 years (range 1–15) HSCT ± TBI: mean 5 years (range 2–15)	26 Chemo +/- RT 26 HSCT: 17 BuCy, 9 TBI	Growth, cardiac and renal functions were comparable between the two groups. After HSCT: ovarian failure 67% vs. 0% after chemo only.	Leahey et al, 1999.
33	≥1.5 year Chemo: Median 4 (range 1–8) HSCT: Median 7 (range 2–9)	25 Chemo only 8 HSCT: 7 TBI, 1 BuCy	After HSCT: Growth failure (90%), thyroid disorders (40%) cardiac disorders (30%), cataracts (80%), signs of gonadal damage (90%). After Chemotherapy alone: no endocrinopathies but cardiac (30%), renal (8%), and hearing (16%) abnormalities.	Liesner et al, 1994.

**Table 3.** Studies comparing late effects after treatment with HSCT versus without HSCT for childhood AML. Abbreviations: RT indicates radiotherapy; HSCT, hematopoietic stem cell transplantation; HRT, hormone replacement therapy; HRQL, health-related quality of life; Bu, busulphan; Cy, cyclophosphamide; TBI, total body irradiation.



## **2 AIMS OF THE STUDY**

The specific aims of the thesis were:

1. To determine the spectrum of chronic toxicities in long-term survivors of pediatric allogeneic HSCT
2. To compare the chronic sequelae after various conditioning regimens (sTBI, fTBI, chemotherapy)
3. To compare outcomes after allo-HSCT in first complete remission (CR1) versus after allo-HSCT in second complete remission (CR2) or more advanced disease
4. To assess how much additive long-term toxicity is associated with allo-HSCT in the treatment of childhood AML





### 3 PATIENTS AND METHODS

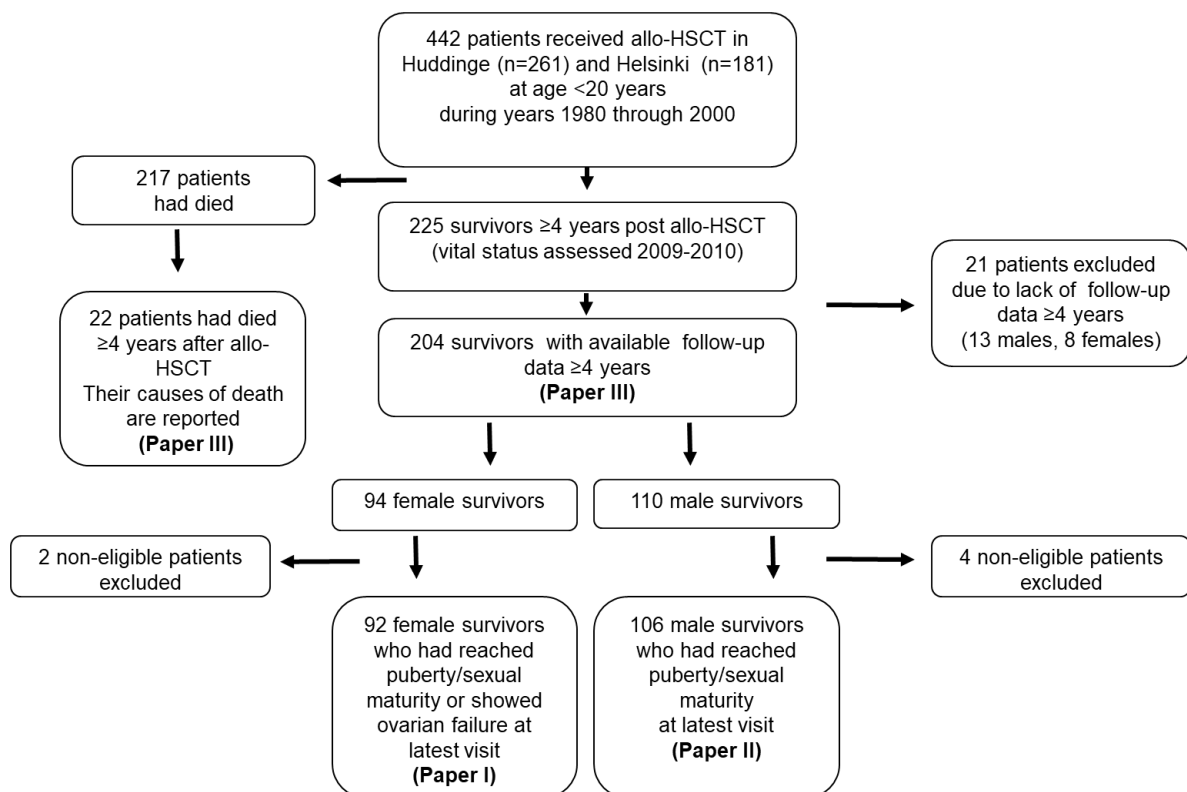
The following methods were applied in this thesis. More detailed information can be found in the individual papers.

#### 3.1 LONG-TERM SURVIVORS OF ALLOGENEIC HSCT (PAPERS I-III)

A retrospective case-note review: Prospectively collected data from high-quality medical records was retrospectively reviewed.

##### 3.1.1 Study population

All children treated with HSCT at the age of 1–19 years in Huddinge and Helsinki during 1980–2000, and alive at least 4 years after HSCT with available medical records were included. The flow chart of the study population in **Papers I–III** is presented in Figure 3. The main characteristics of the whole cohort (n=204) described in **Papers I–III** are summarized in Table 4.



**Figure 3.** Flow-chart. Study population in Papers I–III. 204 (91%) of the 4-year survivors of pediatric allogeneic HSCT transplanted in Huddinge and Helsinki 1978–2000.

<b>Characteristics of the study population in Papers I-III.</b>	
<b>Total material</b>	<b>204</b>
Female, n (%)	94 (46)
Male, n (%)	110 (54)
Median age at HSCT (range), years	8 (0.4-19)
Median age at latest visit (range), years	20 (8.5-42)
Median follow-up time (range), years	12 (4-28)
<b>Diagnosis</b>	<b>n (%)</b>
ALL	78 (38)
AML	46 (23)
SAA	31 (15)
Other	49 (24)
<b>Conditioning</b>	<b>n (%)</b>
sTBI	59 (29)
fTBI	86 (42)
Busulfan	30 (15)
Cy only	23 (11)
TNI	6 (3)
<b>Donor</b>	<b>n (%)</b>
Related	135 (66)
Unrelated	69 (34)
<b>Chronic GvHD</b>	<b>n (%)</b>
No	151 (74)
Yes	51 (25)
Unknown	2 (1)
<b>Patients with leukemia, n</b>	<b>124</b>
Cranial irradiation for leukemia, n (%)	26 (13)
Testicular irradiation for leukemia, n (%)	8 (4)
Remission status of leukemia	n (%)
CR 1	63 (51)
CR 2-4	61 (49)

**Table 4.** Characteristics of the 204 survivors of pediatric allogeneic HSCT transplanted in Huddinge and Helsinki during the years 1978 through 2000 included in Papers I–III. ALL, acute lymphoblastic; AML, acute myeloid leukemia; Cy, cyclophosphamide; CR, complete remission; GvHD; graft versus host disease HSCT, indicates hematopoietic stem cell transplantation; TBI, total body irradiation; sTBI, single fraction TBI; fTBI, fractionated TBI; and TNI, total nodal irradiation. Adapted from Paper III, Bone Marrow Transplantation (2015) 50, 850–857.

**Paper I:** All adult/pubertal female survivors (n=92) who were late pubertal/post-pubertal or showed signs of ovarian failure at their latest visit were included.

**Paper II:** All male survivors (n=106) who were late pubertal or post-pubertal at latest visit were included.

**Paper III:** All survivors (n=204) with follow-up data of 4 years or more were included regardless of their pubertal status at latest visit.

### 3.1.1 Treatment characteristics

The University Hospital in Huddinge used sTBI 10-12 Gy combined with CY (120 mg/kg) between 1978-1995. From 1993 fTBI was given as 12 Gy in four fractions. The Helsinki University Hospital used sTBI from 1978 till 1983. From 1984 TBI was given as fTBI 10-12 Gy in 5-6 fractions.

Patients with SAA received conditioning with Cy (200 mg/kg) either without or in combination with total lymph nodal irradiation (TNI) 6 Gy or fTBI (10Gy). Bu (16 mg/kg) was given in combination with Cy (100-200 mg/kg) to patients with immunodeficiency or inborn errors of metabolism. Patients with ALL and AML had received treatment according to the common NOPHO-protocols prior to allo-HSCT.

### 3.1.2 Methods

#### *Follow-up procedures and data collection*

Time from allo-HSCT to the last recorded visit was determined as follow-up time. Annual visits included clinical and laboratory examinations. Every medical event or chronic health condition occurring after allo-HSCT until the most recent visit was noted. Medical records were reviewed in detail for data on medications and hormone replacement therapies (HRTs) both current and ever used (growth hormone, thyroxin and estrogen or testosterone), presence of cGVHD (limited or extensive) during follow-up, and data from growth charts were collected.

For **Paper I**, data were collected on pubertal development, menarche and pregnancies. For **Paper II**, data on results from sperm analyses, pubertal development and pregnancies in partners were collected. Adverse health events (AE) were graded retrospectively according to CTCAE v3.0 (Figure 4). Only limited data could be obtained through the medical records on neurocognitive and psychological AEs. Causes of late deaths (more than 4 years after allo-HSCT) were analysed separately.

				<b>Grade 5 AE</b>
			<b>Grade 4 AE</b>	
	<b>Grade 3 AE</b>			
	<b>Grade 2 AE</b>	<b>Severe</b>	<b>Life-threatening or disabling</b>	<b>Death</b>
<b>Grade 1 AE</b>	<b>Moderate</b>			
<b>Mild</b>				

**Figure 4.** Grading of adverse health events (AEs).

### 3.1.3 Statistical methods

All data in **Papers I** and **II** are reported as mean  $\pm$  standard deviation (SD) and range and in **Paper III** as median and range. The SPSS statistical software, version 20, was used for all the statistical analyses in **Papers I–III**.

In **Papers I–III** Mann-Whitney U test was used for continuous variables, and  $\chi^2$  test and Fisher's exact were used for categorical variables. Bi- and multivariate logistic regression were used for calculating odds ratios (ORs) with 95% confidence intervals. The continuous variables included age at HSCT, follow-up time from HSCT, and serum levels of FSH and LH. In the regression analysis, categorical predictors included dummy variables (0/1) prepubertal at HSCT, Leukemia diagnosis, SAA diagnosis, conditioning with TBI, and cGVHD. The categorical predictors remission status at HSCT, CRT, and in male leukemia survivors, testicular irradiation.

In **Paper II**, for predicting any spermatozoa in the seminal fluid by using testicular size and 1/FSH receiver operating characteristics (ROC) curves were constructed where standard error of the area under the curve (AUC) was estimated by using nonparametric distribution of parameters. In order to test adult testicular volumes and serum gonadotropins as dichotomous dependent and independent variables the following cut-offs were used: 15 mL for testicular volumes (the normal lower 2SD value) and 10 IU/mL for serum gonadotropins.

In **Paper III**, explanatory covariates were selected by using Pearson correlation for the step-wise forward regression analysis for modeling the correlation of dependent variables (the total number of non-hormonal chronic health conditions and severity of chronic health conditions, multiple hormonal substitutions) with the explanatory covariates sex, pubertal status at transplant, age at transplant, cGVHD, follow-up time, diagnosis group (SAA, leukemia, Others) and conditioning regimen (sTBI, fTBI, TBI, Cy and Bu). Additional covariates used only for the Leukemia group were cranial and testicular irradiation, and remission status at HSCT.

No corrections were made for multiplicity. A p-value of  $<0.05$  was set to indicate statistical significance in all papers.

### 3.1.4 Ethical considerations

The studies were approved by the Regional Ethical Review Board in Stockholm and the Research Ethics Committee of the Helsinki University Central Hospital.

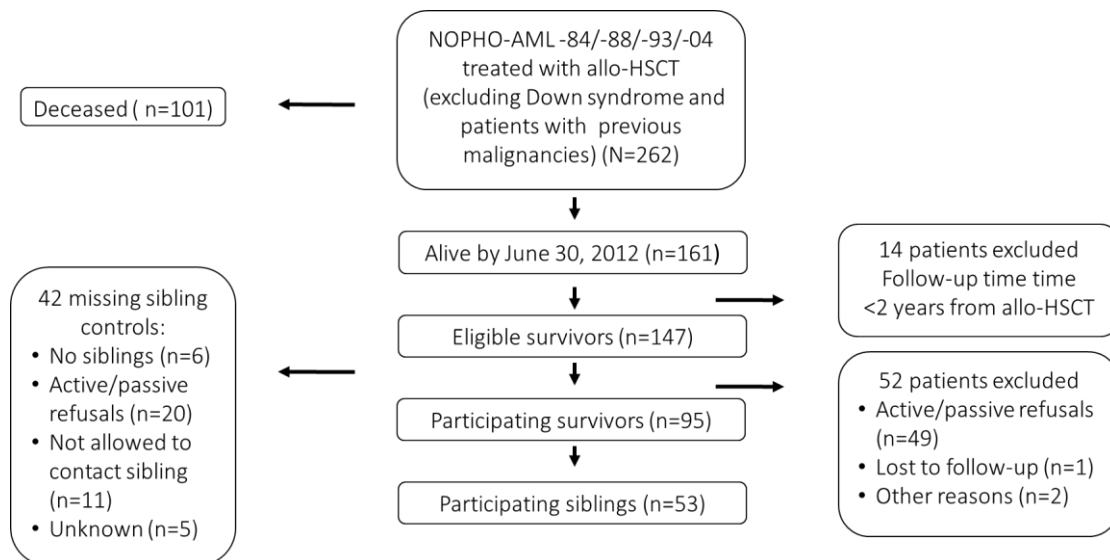
## 3.2 LONG-TERM SURVIVORS OF CHILDHOOD AML TREATED WITH ALLO-HSCT (PAPER IV)

### 3.2.1 Study design

A cross-sectional study. The questionnaire data was collected during 2012–2013.

### 3.2.2 Study population

All 2-year survivors of childhood AML treated with the NOPHO-AML-84, -88, -93 or 2004 protocols with allo-HSCT at Nordic transplantation units in Sweden, Finland, Norway and Denmark when younger than 21 years were identified through the NOPHO-AML database. Patients with previous malignancies or Down syndrome were not included. They were mailed a questionnaire and invited to participate in the study. Whenever possible, a sibling of an allo-HSCT survivor closest in age was asked to participate. In total, 95 out of the eligible 147 survivors completed the questionnaire and 53 of them had a sibling control. The flow chart of the study population in **Paper IV** is shown in Figure 5.



**Figure 5.** Flowchart of patients from the NOPHO-AML-84, -88, -93 and -04 trials included in the AML allo-HSCT group alive on June 30th, 2012.

### 3.2.3 Methods

Basic background on disease status, treatment and cGVHD was retrieved from the NOPHO-AML database and from the treating transplant centers. The questionnaire included 130 questions on health, use of medications, medical conditions, physical health, activities of daily living, education, marital status, smoking, anxieties and concerns related to previous AML treatment. With the exception of six additional questions related to allo-HSCT, questions were part of the validated questionnaire from the Childhood Cancer Survivor Study ([www.ccss.stjude.org](http://www.ccss.stjude.org)) (113) and the questionnaire was identical to the one that was completed previously by the control group of 101 AML survivors treated with the NOPHO-AML-84, -88 or -93 protocols but without allo-HSCT (109). The second control group were siblings (n=53) of participating allo-HSCT survivors, if there were several siblings the sibling

closest in age was invited to participate. The sibling questionnaire was identical with the exception of 15 AML- or allo-HSCT related questions that were omitted. Two reminders were sent both to the allo-HSCT survivors and siblings.

### **3.2.4 Statistical analyses**

The data are reported as median and range. Categorical outcomes were compared by using Fisher's exact test. For continuous variables Mean's median test was used. Logistic regression analysis adjusted for gender, age and time-from-diagnosis was used when comparing outcomes between the two AML survivor groups. The results are reported both as crude and adjusted ORs. In sibling pair comparisons conditional logistic regression was used and exact logistic regression was used when the outcomes were too scarce and adjusting for confounders could not be performed. Dummy variables (0/1) for CR status, gender, cGVHD, age at allo-HSCT  $\geq 10$  years, follow-up time  $\geq 10$  years, TBI, underweight and any chronic grade  $\geq 3$  condition were used in the uni- and multivariable regression analyses. Age at questionnaire was included as a continuous variable. A p-value of  $<0.1$  in the univariable analyses was required for inclusion in the multivariable analyses. P-values  $<0.05$  were considered significant. No corrections were made for multiplicity.

### **3.2.5 Ethical considerations**

The national ethical boards in Sweden, Finland, Norway and Denmark approved the study according to the national regulations. The participants filled in a written informed consent.

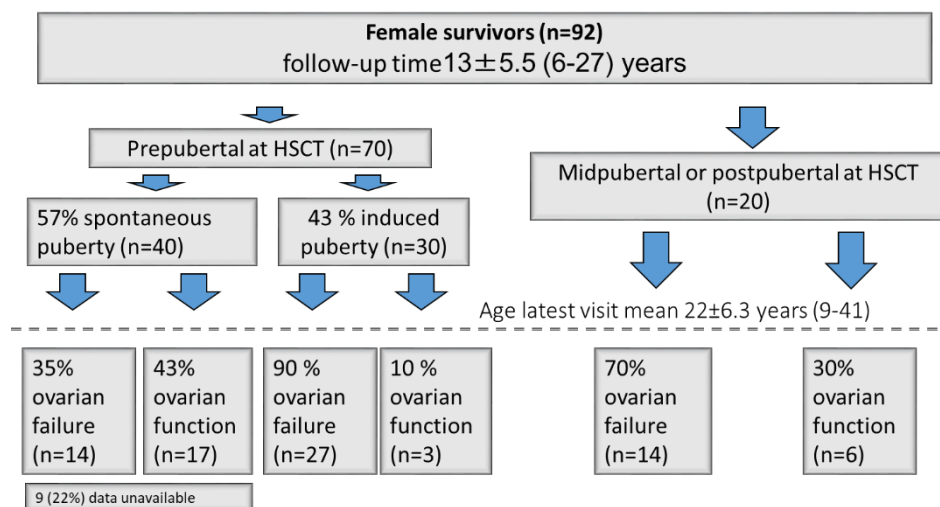
## 4 RESULTS

### 4.1.1 Ovarian failure and premature menopause (I)

Altogether 92 female survivors who were late or post-pubertal or had ovarian failure by latest visit were included in the analyses. Their mean age at latest visit was  $22\pm6$  (range 8–40) years and mean follow-up time after allo-HSCT was  $13\pm5.5$  (range 6–27) years.

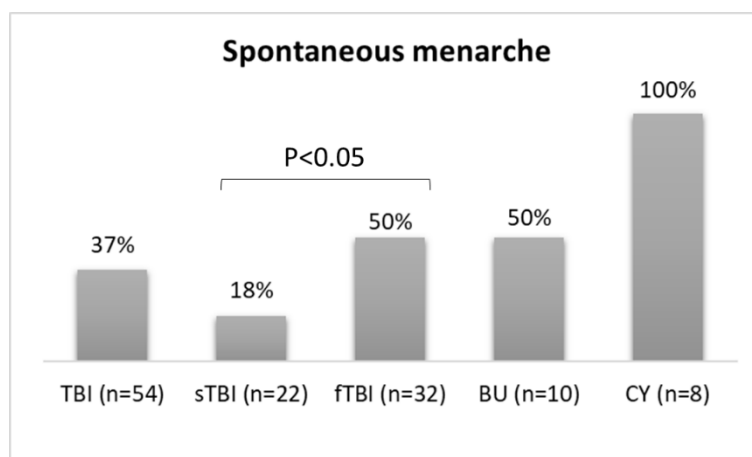
At the time of allo-HSCT, 70 (76%) were prepubertal (Tanner 1), 12 (13%) mid-pubertal (Tanner 2–3) and 8 (9%) late or post-pubertal (Tanner 4–5). Out of the 92 included female survivors 71 (77%) had been conditioned with TBI-based, 10 (11%) with Bu-based and 10 (11%) with Cy-based conditioning regimens. Forty-two (46%) had received fTBI and 29 (32%) sTBI, and one girl had received TNI as the only form of irradiation. Twenty-six (28%) of the female survivors had cGVHD.

Forty out of the 70 girls who were prepubertal at allo-HSCT had spontaneous pubertal development and 30 had spontaneous menarche. Out of the 40 women who entered puberty spontaneously, 35% had entered premature menopause by their latest visit. Almost all (90%) of the thirty prepubertal girls without spontaneous pubertal development had ovarian failure at their latest visit (Figure 6). More than 70% of all the female survivors who had received TBI or Bu experienced ovarian failure by their latest visit.



**Figure 6.** Spontaneous onset of puberty and ovarian function evaluated at the latest visit among the 92 female survivors of allo-HSCT.

A total of 30 (43%) of the prepubertal girls and three (25%) of the mid-pubertal girls had spontaneous menarche. All the girls without prior cytotoxic therapy who had received Cy-based conditioning regimens for SAA had both spontaneous puberty and menarche. A higher proportion of the girls who had received Bu-based conditioning compared to TBI had spontaneous menarche; however, there was no significant difference when comparing Bu to fTBI (Figure 7).



**Figure 7.** Proportion of the girls with spontaneous menarche (n=33) within different conditioning groups. Only the girls who were prepubertal or mid-pubertal at allo-HSCT with no menarche prior to allo-HSCT are included in the numbers (n=72).

The chance for spontaneous menarche was significantly higher if the patient had been transplanted for SAA, had not received TBI nor received treatment for leukemia, and had lower serum FSH and LH levels (Table 5). None of the survivors who had received CRT prior to allo-HSCT had spontaneous menarche. They had all been treated for ALL and received TBI-based conditioning regimens. sTBI was identified as the strongest predictor for needing HRT at latest visit (Table 5).

Predictors	OR	95%CI	p-value
<b>Lack of spontaneous puberty</b>			
Age at HSCT	1.2	1.0–1.4	0.015
<b>Lack of spontaneous menarche</b>			
Diagnosis other than SAA	6.1	1.3–31	0.030
TBI	5.2	1.6–17	0.006
Leukemia	3.6	1.3–9.7	0.011
Age at HSCT	1.1	1.0–1.3	0.06
Increase in FSH (1 IU/l)	1.035	1.01–1.1	0.002
Increase in LH (1 IU/l)	1.09	1.03–1.1	0.001
<b>Estrogen HRT at latest visit</b>			
sTBI	4.3	1.3–14	0.016
SAA	0.2	0.1–0.9	0.033

**Table 5.** Predictors for lacking spontaneous puberty or menarche, and the need of estrogen replacement therapy. Results from bivariate logistic regression analysis. Table modified from original Paper I. Reprinted with permission of © 2014 European Society of Endocrinology.

Out of the 92 female survivors, 86 (93%) were 15 years or older at latest visit. Ten (12%) of these 86 women had had 14 recorded pregnancies. Two of the pregnancies (14%) had led to a miscarriage. Twelve children had been born, of which three (25%) were born preterm. Four women with recorded pregnancies had received ftTBI-, three Bu- and two Cy-based conditioning regimens. One woman with SCID had received sTBI with ovarian shielding and given birth to two children; both were born full-term and healthy.



#### 4.1.2 Recovery of spermatogenesis and testicular volumes (II)

Altogether 106 male survivors were late pubertal or post-pubertal at latest visit and were included in the analyses. The mean age of the late/post-pubertal male survivors at latest visit was  $22\pm6$  (range 12–42) years and mean follow-up time after allo-HSCT was  $13\pm4.8$  (range 4–28) years.

When receiving allo-HSCT, 82 (77%) were prepubertal (Tanner 1), 19 (18%) mid-pubertal (Tanner 2–3) and 5 (5%) late or post-pubertal (Tanner 4–5). Out of these 106 included male survivors, 71 (67%) had been conditioned with TBI-based, 18 (17%) with Bu-based and 17 (16%) with Cy-based conditioning regimens. Five male survivors had received TNI. Twenty-five (24%) of the male survivors had cGVHD.

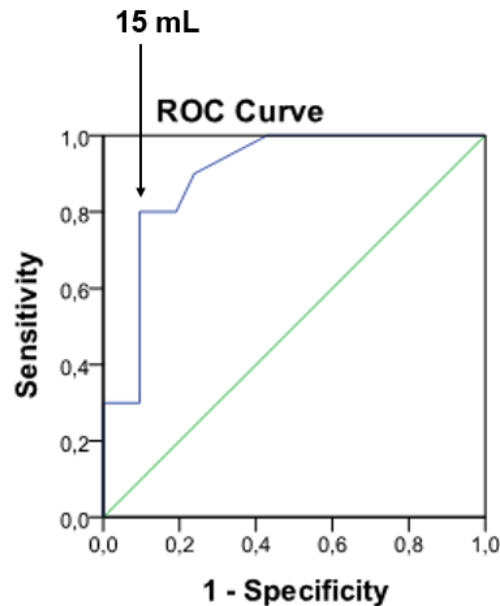
Out of the 82 males who were prepubertal at transplantation, 68 (83%) had spontaneous pubertal development. The only factor that significantly decreased the likelihood of spontaneous puberty was testicular irradiation given as part of the ALL treatment. At latest follow-up visit, 28 (26%) males were on testosterone replacement therapy. Serum gonadotropin values were available for 100 survivors. Leukemia patients had higher FSH levels than survivors treated for SAA and other disease indications ( $p<0.001$  and  $p<0.01$ , respectively). Recipients of TBI-based conditioning regimens had higher FSH levels compared to recipients of non-TBI-based regimens ( $p<0.01$ ) and LH levels were significantly higher after sTBI compared to fTBI ( $p<0.05$ ). In our data, we did not see any significant correlation between the gonadotropin levels and cGVHD.

Altogether 31 (29%) male survivors had had a semen analysis performed. Spermatozoa were detected in 10 (32%) of the samples. Factors that predicted active spermatogenesis were: a diagnosis other than leukemia, a testicular volume 15 mL or above, conditioning without TBI and FSH levels below 10 IU. The results from bi- and multivariate analyses are shown in Table 6.

	OR	Bivariate 95% CI	P	OR	Multivariate 95% CI	P
<b>Adult testicular volume &lt; 15mL</b>						
TBI	15	4.0–59	<0.001	15	4.0–59	<0.001
Leukemia	4.9	1.5–17	<0.01			
FSH > 10 IU	1.1	1.0–1.2	<0.04			
<b>Active sperm production</b>						
No leukemia diagnosis	17	2.6–113	<0.003	20	1.9–210	<0.01
Testicular volume $\geq$ 15 mL	14	2.1–98	<0.007	17	1.4–216	<0.03
No TBI	30	2.8–322	<0.005			
FSH < 10 IU	0.8	0.7–1.0	<0.047			
<b>Testosterone substitution</b>						
TBI	9	2.0–41	<0.004	7.8	1.7–36	<0.009
Prepubertal at HSCT	4.8	1.0–11	<0.04	7.2	1.3–41	<0.03
Testicular irradiation	11	2.1–58	<0.005	10	1.5–68	<0.02
Leukemia	5.4	1.7–18	<0.004			
CRT for leukemia	3.5	1.1–11	<0.03			
No spontaneous puberty	5.4	1.6–18	<0.007			

**Table 6.** Predictors for adult testicular volume <15 mL, active sperm production, and the need of testosterone substitution after allogeneic HSCT in childhood and adolescence. Only the significant values are indicated. Modified from original Paper II. Reprinted with permission of ©2014 Wiley Periodicals, Inc,

Testicular volumes were less affected by chemotherapy-based conditioning regimens. Testicular volumes above 15 mL predicted spermatozoa in sperm samples with 80% sensitivity and 91% specificity (Figure 8).



**Figure 8.** Receiver operating characteristic curve (ROC) for testicular volume (AUC 0.89, n=31) detecting spermatozoa in seminal fluid. The sensitivity and specificity for cut-off value 15 mL is indicated by an arrow. Modified from original Paper II, reprinted with permission of ©2014 Wiley Periodicals, Inc,

None of the fifteen survivors with testicular volumes above 15 mL had received sTBI but five of them had been conditioned with fTBI. Two of the 10 patients who had recovered their spermatogenesis had been treated for acute leukemia and their testicular volumes were 15mL and 25 mL; both had received fTBI and neither of them had cGVHD. The other eight survivors with detectable spermatozoa had been treated for SAA (n=4), chronic granulomatous disease (n=1), CML (n=1), thalassemia (n=1), and myelodysplastic syndrome (n=1). The median testicular volume in survivors with spermatozoa was 17 (range 10–35) mL, four had received fTBI but none of them had received sTBI or testicular irradiation. Eight survivors with small adult testicular volumes ( $\leq 4$  mL) had all been treated for acute leukemia, and six (80%) of them had been conditioned with sTBI and two (20%) with fTBI. Three of them had received testicular irradiation as well. Sperm samples (n=2) from this group showed no detectable spermatozoa.

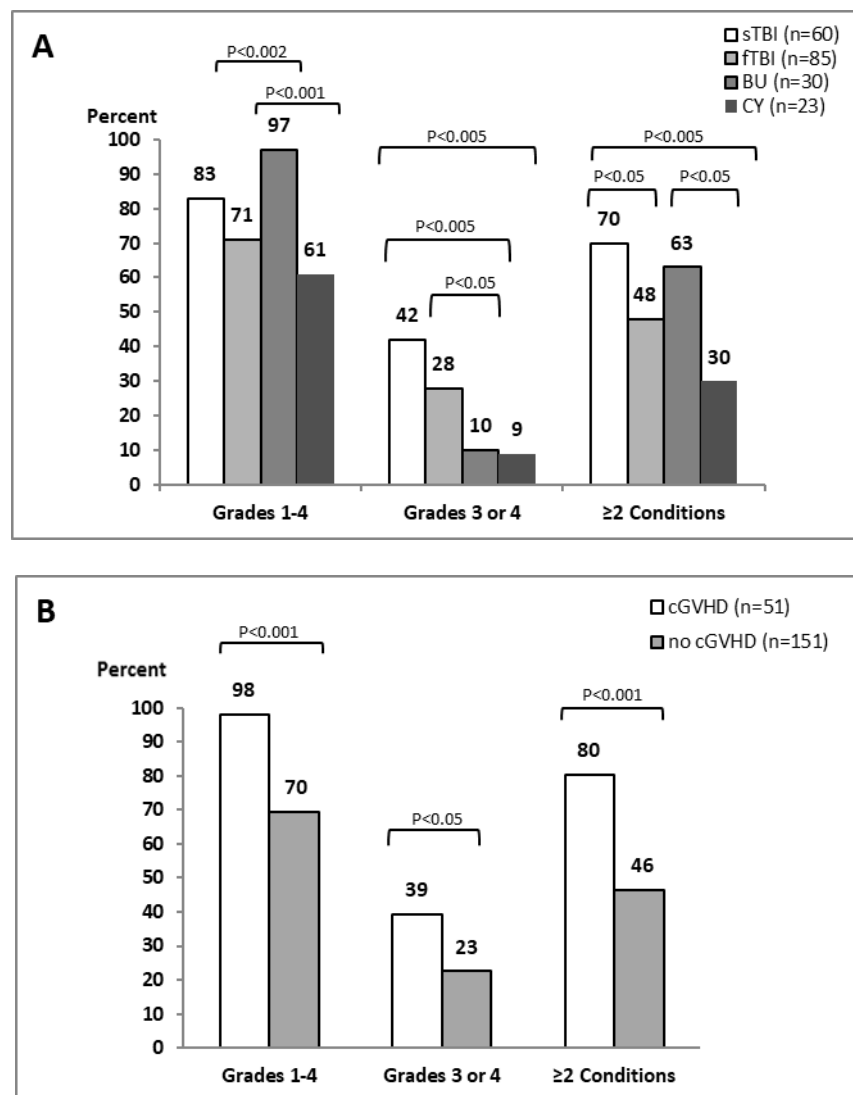
To our knowledge only two out of the 106 male survivors in our cohort had fathered offspring. One had been treated for SAA with Cy-based conditioning and the other had been treated for chronic myeloid leukemia and conditioned with fTBI (10 Gy) with no anti-leukemia treatment given prior to allo-HSCT.

### 4.1.3 Adverse events and late mortality (III)

Late effects were determined through medical records in 204 (91%) of the cohort of pediatric allogeneic HSCT survivors transplanted in Huddinge and Helsinki 1978–2000. The median follow-up time was 12 (range 4–28) years after allo-HSCT.

#### *Non-hormonal chronic conditions*

Almost all (84%) of the survivors had at least one chronic non-hormonal adverse event (AE). TBI-based conditioning associated with the highest burden of late toxicities and sTBI was more toxic than fTBI. However, there was also considerable morbidity associated with the Bu-based conditioning regimens and practically all (97%) Bu-conditioned survivors had at least one non-hormonal chronic condition (Figure 9A).



**Figure 9 A-B** Non-hormonal adverse events graded according to CTCAE v3.0 excluding cataracts. Cumulative incidence of chronic health conditions among survivors (A) after TBI-based conditioning regimen compared with BUu- based and Cy-based conditioning regimens and (B) among survivors with and without cGVHD. The number of patients included in each group and significant P-values are given. Adapted from original Paper III, Bone Marrow Transplantation (2015) 50, 850–857. Reprinted with permission of ©Springer Nature.

However, survivors conditioned with Bu had significantly fewer severe (grade 3 or higher) conditions compared to the survivors conditioned with sTBI and fTBI (10% vs. 42% and vs. 28%, respectively). cGVHD was identified as a major risk factor for non-endocrine chronic conditions. Patients with cGVHD had both higher numbers (98% vs 70%,  $p>0.001$ ) and more severe (grade 3 or higher) (80% vs. 46%,  $p>0.001$ ) non-endocrine chronic conditions compared to patients without cGVHD (Figure 9B). Severe grade 3 or 4 conditions had been diagnosed in half (50%) of the survivors with a follow-up time of 20 years or more (shown in Appendix: **Paper III**, Figure 1D).

### *Endocrine disorders*

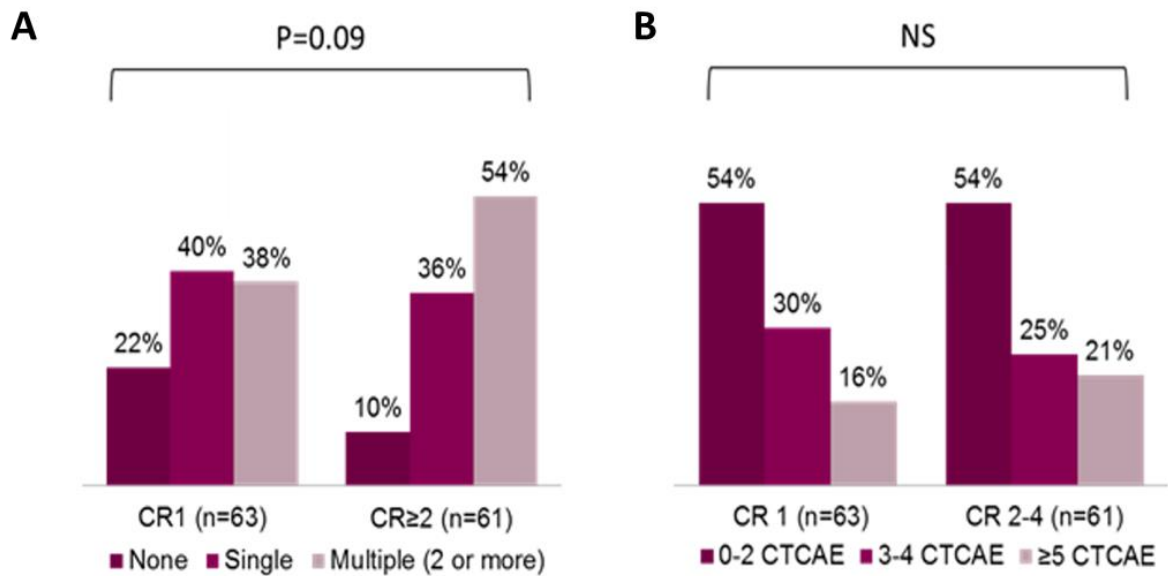
TBI-recipients had the highest prevalence of endocrine disorders: growth hormone deficiency had been diagnosed in 67%, hypothyroidism in 46%, and 62% of the survivors received HRT with either testosterone or estrogen. The prevalence of growth hormone deficiency and HRT use was significantly higher compared to Bu while the difference in hypothyroidism was not significant. Among Cy-conditioned survivors only two had hypothyroidism and two were on HRT (Table 7). Physical growth was less impacted by the chemotherapy-based conditioning regimens. Extremely short statures were seen mainly after TBI-based conditioning regimens and in leukemia survivors. The Cy-conditioning group consisted of patients treated for SAA while the Bu-conditioning group included many patients with inborn errors of metabolism, and in some of those cases height SDS was improved after allo-HSCT.

	sTBI (n=60) n(%)	fTBI (n=85) n(%)	sTBI vs. fTBI p-value	TBI (n=145) n(%)	BU (n=30) n(%)	TBI vs. BU p-value	BU (n=30) n(%)	CY (n=23) n(%)	BU vs. CY p-value
Growth hormone deficiency	40 (67)	29 (34)	<0.001	69 (48)	7 (23)	<0.02	7 (23)	0 (0)	<0.02
Thyroxine deficiency	39 (65)	28 (33)	<0.001	67 (46)	8 (27)	0.07	8 (30)	2 (9)	NS
Testosterone or estrogen HRT	40 (67)	50 (59)	NS	90 (62)	9 (30)	<0.002	9 (30)	2 (9)	0.09

**Table 7.** Hormone deficiencies and hormonal substitutions within the different conditioning groups.

### *The impact of remission status at transplantation*

The remission status at allo-HSCT did not significantly impact number of hormonal substitutions or number of chronic health conditions in leukemia survivors (Figure 10). However, leukemia survivors transplanted in CR 2 or higher needed more frequently HRT with estrogen or testosterone.



**Figure 10.** The impact of CR status (A) on the number of needed hormonal substitutions and (B) on the prevalence of non-endocrine chronic conditions among allo-HSCT survivors treated for leukemia. CR1 indicates allo-HSCT in first complete remission; CR≥2, allo-HSCT after relapse; CTCAE, adverse event graded according to the CTCAE version 3.0.

### *Secondary malignancies*

Altogether thirteen patients among the 247 allo-HSCT patients who had survived at least 4 years after allo-HSCT had been diagnosed with a secondary malignancy. The secondary malignancies included malignant mesothelioma (n=1), malignant melanoma (n=1), malignant meningiomas (n=1), renal carcinoma (n=1), papillary thyroid carcinoma (n=1), secondary AML (n=2), ALL (n=1), breast cancer (n=1), oligodendroglioma (n=1), basal cell carcinoma (n=1), and oral cancer (n=1), and one patient had an unspecified second malignancy. Only survivors conditioned with TBI had been diagnosed with secondary malignancies with the exception of one patient with SAA who died 12 years after allo-HSCT due to oral cancer.

### *Causes of late deaths*

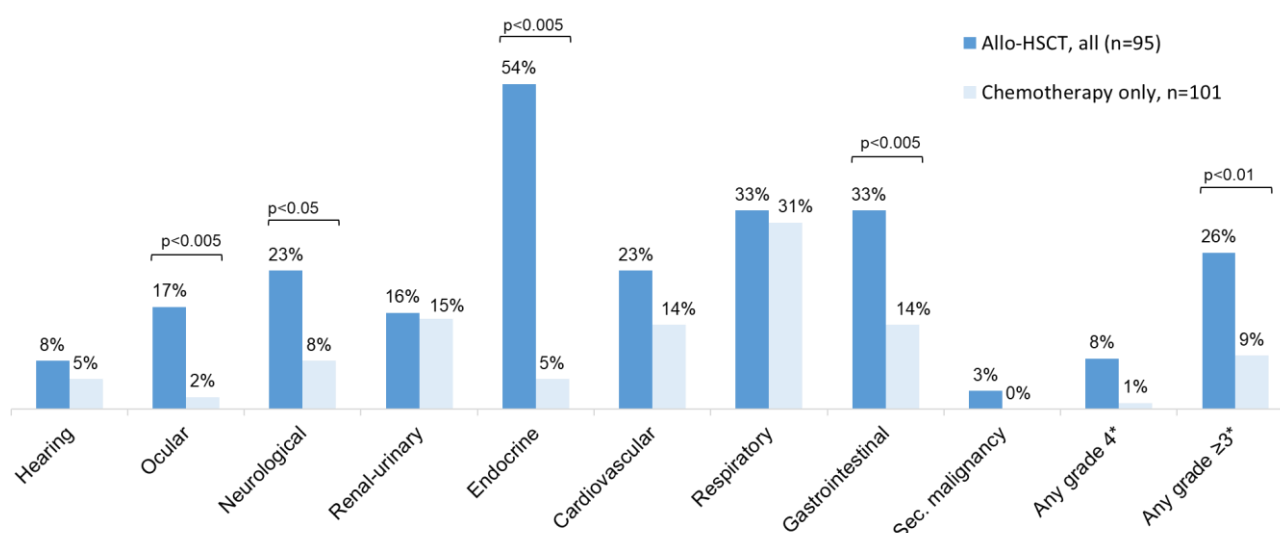
Twenty-two patients died late, more than 4 years after HSCT, between 5 and 24 years after allo-HSCT at a median age of 17 (range 10–36) years. Eight (36%) of them died due to complications related to extensive cGVHD. All the causes of late deaths are listed in Table 8.

Late causes of death (n=22)	Years from HSCT	n
	Median (range)	
Chronic GVHD		
Pulmonary cGVHD	8 (6-13)	7
Gastrointestinal cGVHD	10 and 13	2
Secondary malignancy	10 (9-16)	5
Relapse	6 (5-12)	3
Infection	6 (6)	2
Cardiovascular	22 and 24	2
Primary diagnosis	18	1

**Table 8.** Causes of late deaths ( $\geq 48$  months after HSCT) by the year 2010 after pediatric allo-HSCT at Huddinge and Helsinki University Hospitals between 1978 and 2000. Modified from original Paper III with permission of ©Springer Nature.

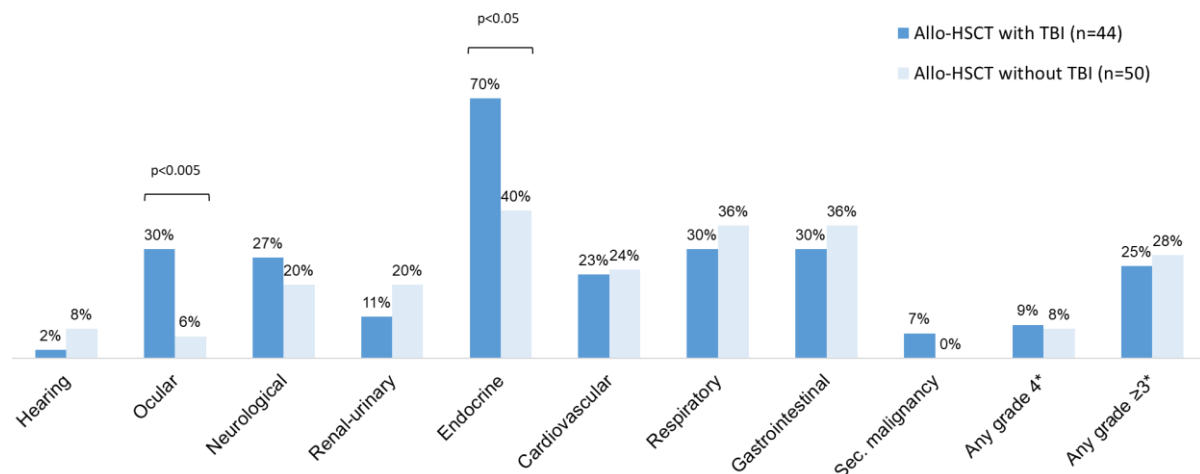
#### 4.1.4 Additive toxicity associated with allo-HSCT in childhood AML treatment (IV)

The AML survivors who had been treated with allo-HSCT reported significantly more health disorders (Figure 11) and more frequent use of medications than the AML survivors treated with chemotherapy only. The medications used more frequently by allo-HSCT survivors during the past two years included agents for thyroid disorders (hypothyroidism) (21% vs. 0%,  $p < 0.001$ ), cardiovascular conditions (10% vs. 1%,  $p < 0.05$ ), analgesics (32% vs. 11%,  $p < 0.01$ ) and nutritional supplements (36% vs. 8%,  $p < 0.001$ ), and a higher proportion of the female survivors aged  $> 14$  years used estrogen (51% vs. 9%,  $p < 0.001$ ).

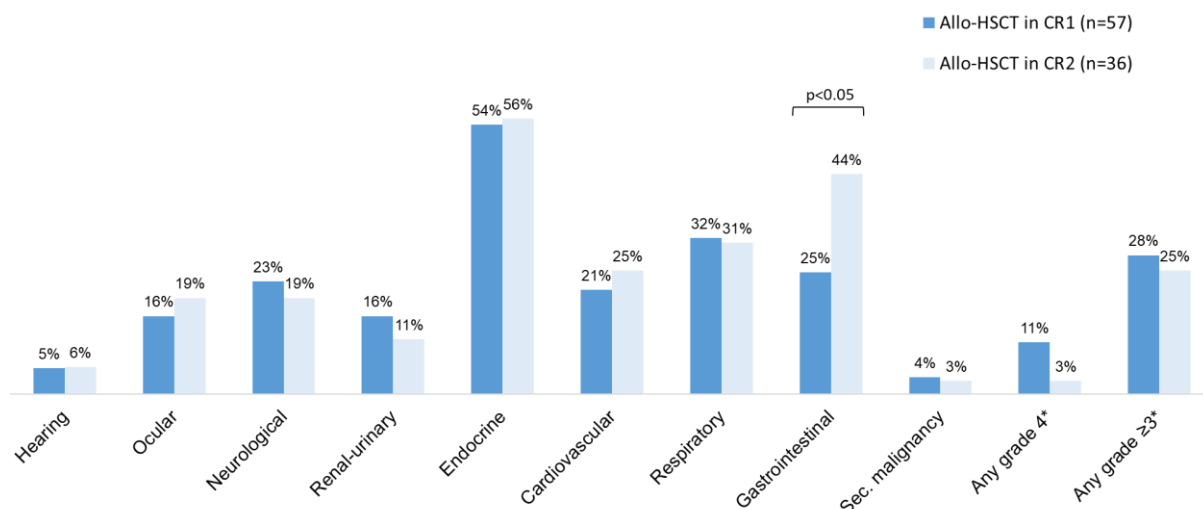


**Figure 11.** The proportion of childhood AML survivors treated with allo-HSCT (n=95) and without allo-HSCT (n=101) reporting at least one disorder in an organ system, and any severe grade  $\geq 3$  or grade 4 condition as graded by CTCAE version 3.0 \* excluding gonadal failure and infertility. P-values were calculated with logistic regression adjusted for sex, age and time from diagnosis. Only significant p-values are indicated.

TBI associated with reporting more endocrine disorders and ocular complications (Figure 12). Survivors who received allo-HSCT after relapse in CR2 reported more gastrointestinal disorders compared to survivors transplanted in CR1 (Figure 13). More survivors transplanted in CR2 had cGVHD compared with survivors transplanted in CR1 (42% vs. 32%, NS). The prevalence of cGVHD was significantly higher among allo-HSCT survivors conditioned with Bu compared with TBI (44% vs. 16%,  $p<0.005$ ).



**Figure 12.** The proportion of childhood AML survivors treated with allo-HSCT including TBI (n=44) and without TBI (n=50) reporting at least one disorder in an organ system, and any severe grade  $\geq 3$  or grade 4 condition as graded by CTCAE version 3.0 \* excluding gonadal failure and infertility. P-values were calculated with unadjusted logistic regression. Only significant p-values are indicated.



**Figure 13.** The proportion of childhood AML survivors who received allo-HSCT in CR1 (n=57) and in CR2 (n=36) reporting at least one disorder in an organ system, and any severe grade  $\geq 3$  or grade 4 condition as graded by CTCAE version 3.0 \* excluding gonadal failure and infertility. P-values are calculated with unadjusted logistic regression. Only significant p-values are indicated.

Survivors transplanted with Bu-based conditioning in CR1 had an increased OR for reporting endocrine disorders and gastrointestinal disorders when compared with survivors treated with chemotherapy only remaining in CR1. In addition, survivors transplanted after relapse (in CR2) and conditioned with Bu had a significantly increased OR (OR 3.6, 95% CI 1.1–12) for reporting cardiovascular disorders when compared with survivors treated with chemotherapy only remaining in CR1 (Appendix: Paper IV, Figure 3B).

### *Limitations in physical activities*

A higher proportion of the allo-HSCT survivors experienced limitations in physical activities when compared to the conventionally treated AML survivors as well as sibling controls (39% vs. 7% and vs. 9%, respectively). In multivariable analysis (among allo-HSCT survivors only), the factors that significantly increased the OR for reporting limitations in vigorous activities (such as lifting heavy objects, running and strenuous sports) were the presence of cGVHD, allo-HSCT after relapse in CR2, being underweight and the presence of any severe grade  $\geq 3$  chronic condition (Table 9). The OR for reporting limitations in moderate physical activities (such as pushing a vacuum cleaner, moving a table or carrying groceries) was increased by being underweight (BMI-z-scores  $<2SD$  or BMI $<18.5$ ) and by the presence of any grade 3 CTCAE chronic condition (Table 9).

<b>Limitations in vigorous activities, results of multivariable analysis for OR.</b>				
<b>Variable</b>	<b>Category</b>	<b>OR</b>	<b>95%CI</b>	<b>P-value</b>
cGVHD	cGVHD vs. no cGVHD	3.6	1.1, 12	0.04
CR status	CR2 vs. CR1	3.3	1.0, 11	0.05
TBI	TBI vs. no TBI	0.8	0.4, 1.4	0.35
Underweight (BMI SD $<-2$ or BMI $<18.5$ )	Underweight vs. Healthy/Overweight	12	1.4, 101	0.02
Chronic condition grade $\geq 3$	Any grade $\geq 3$ vs. no grade $\geq 3$	12	3.5, 42	$<0.001$
<b>Limitations in moderate activities, results of multivariable analysis for OR.</b>				
<b>Variable</b>	<b>Category</b>	<b>OR</b>	<b>95%CI</b>	<b>P-value</b>
<b>Underweight (BMI SD<math>&lt;-2</math> or BMI<math>&lt;18.5</math>)</b>	Underweight vs. Healthy/Overweight	5.7	1.0, 32	0.048
<b>Chronic condition grade <math>\geq 3</math></b>	Any grade $\geq 3$ vs. no grade $\geq 3$	11	3.2, 40	$<0.001$

**Table 9.** Factors associated with reporting limitations in physical activities among AML survivors treated with allo-HSCT (n=95). Only the explanatory variables with p-values  $<0.1$  in univariable analyses were included in the multivariable analyses shown in the table.

Despite the higher burden of health problems, more than half (57%) of the allo-HSCT survivors rated their health as excellent or very good, while a significantly high proportion (15%) reported having a health problem or an impairment that kept them from attending school or work. Allo-HSCT survivors' employment and marital status did not significantly differ from siblings. Although allo-HSCT survivors were less likely to smoke compared to their siblings and chemotherapy only survivors, as many as 8% of the survivors aged  $\geq 15$  years reported being current smokers.



### *Fertility*

Out of the survivors aged  $\geq 15$  years, 24% of the female and 11 % of the male survivors reported involuntary childlessness for more than a year. The figure among female siblings was similar, 22%, whereas none of the male siblings had experienced involuntary childlessness. Three (8%) of the male survivors 15 years or older reported six natural conceptions in their partners. Ten (27%) of the female allo-HSCT survivors aged  $\geq 15$  years reported a total of 18 pregnancies; 12 of these pregnancies had resulted from natural conception. The miscarriage rate was relatively high (6/17, 35%), but all the children were born reportedly healthy and only three were born prematurely. None of the women had experienced congestive heart failure during or after pregnancy. All the reported pregnancies are listed in Appendix: Paper IV, Table 4.

## 5 DISCUSSION

### 5.1.1 Ovarian failure and premature menopause after pediatric allo-HSCT (I)

The high proportion of female survivors with premature ovarian failure in **Paper I** confirms the ovarian toxicity of alkylating agents and irradiation with ovaries in the field. The majority of the SAA patients retained their ovarian function, indicating that an allo-HSCT with Cy-based conditioning regimen without prior cytotoxic drugs or irradiation does not necessarily compromise fertility, and similar findings have been reported by others (114). Both TBI and Bu were highly gonadotoxic. While we could not show any significant differences in gonadotoxicity between TBI and Bu, ovarian failure tended to be more frequent after sTBI and fTBI. Surprisingly many of the women with reported pregnancies had been conditioned with fTBI while one pregnancy was reported after sTBI in a patient who had not received prior cytotoxic therapy and who received sTBI with ovarian shielding.

We observed that some female survivors had a temporary return of their ovarian function. It would be important not to miss this period of spontaneous ovarian recovery as it can offer an opportunity for fertility preservation methods including oocyte cryopreservation. Also, the patients should be informed of the need of contraceptives to avoid unwanted pregnancies. In non-malignant disease, the collection of ovarian tissue from prepubertal girls with a re-implantation post allo-HSCT can offer a means of retaining normal ovarian function and fertility. In malignant disease the risk of possible contamination with malignant cells limits the use of this technique (115). The risk of contamination has been regarded as high for patients with leukemias, stressing the importance of identifying MRD in the ovarian tissue before re-implantation (116).

All children born to the allo-HSCT female survivors were reportedly healthy at birth, which supports the previous studies that have not shown increased risk of congenital anomalies in offspring born to childhood cancer survivors and transplanted patients (70, 77). Only two miscarriages had been noted in the medical records, in reality the numbers may be higher as it is not certain that patients report miscarriages occurring very early. In this study we also lacked the information on the wish to become a parent and the estimation of fertility rates is not possible. A new evaluation of the same cohort after an additional 10 years might give a more accurate picture of their fertility outcomes.

### 5.1.2 Testicular function after pediatric allo-HSCT (II)

#### *Recovery of spermatogenesis*

In **Paper II**, 10 out of 31 men whose semen sample was analyzed showed spermatogenesis indicating that a long-term recovery of spermatogenesis is possible during extended follow-up. Based on our results the survivors who have received chemotherapy-based conditioning regimens have a higher chance of long-term recovery of spermatogenesis, their serum levels of FSH were more likely to be within normal range and they also had larger adult testicular volumes compared to survivors who had received TBI-based conditioning regimens.

There were, however, even a few survivors who recovered their spermatogenesis after TBI-based conditioning regimens. Our results did not confirm the previous finding that cGVHD is strongly associated with azoospermia (73), this might be due to the study size.

### *Testosterone substitution*

Although Leydig cells tolerate more irradiation and cytotoxic therapy than the germ cells, a significant proportion (26%) of the male survivors received testosterone substitution at the latest follow-up visit. Other studies have also shown that a significant percentage of male survivors may have low testosterone levels ( $< 2\text{ng/mL}$ ), with many male survivors requiring testosterone substitution at a young adult age (63). In **Paper II**, we identified TBI, prepubertal status at HSCT and direct testicular irradiation as the main predictors for needing testosterone HRT.

### *Spermatogenesis*

Based on our results, we propose that measuring adult testicular volume, in addition to measuring FSH levels (117), can provide a useful tool for predicting the potential of recovering spermatogenesis. Eight male survivors who had received TBI with or without direct testicular irradiation at a prepubertal age experienced an arrest in testicular growth with testicular volumes remaining at volumes 4 mL or less. Spermatogenesis occurs in the seminiferous tubules and 80–90% of the bulk of testis is formed by functional seminiferous tubules (118) making the relationship between testicular volume and spermatogenesis significant. Median adult testicular volumes in the Nordic countries vary between 15–23 mL and in our study we set a cut-off at 15 mL. With this cut-off volume, 80% of the survivors with viable sperm could be identified with a 91% specificity. Our results indicate that larger testicular volumes may imply fertility even in patients who have been treated for leukemia or have received TBI. At follow-up, detecting an arrest in testicular growth in pubertal boys may indicate a higher risk of azoospermia. However, a sperm sample is needed for verifying any suspected azoospermia.

### *Fertility preservation*

In vitro fertilization and intracytoplasmic sperm injection can offer a chance of fathering offspring even with low sperm counts (119). Cryopreservation of sperm should be offered to all pubertal and post-pubertal boys prior to cytotoxic therapies. For prepubertal boys, the fertility preservation options are at an experimental level. In non-malignant disease re-implantation of cryopreserved testicular tissue or cells might be a useful fertility preservation approach while in malignant disease it includes a risk of malignant contamination (120). For prepubertal boys, in-vitro differentiation of germ cells combined with techniques for fertility preservation might offer a fertility preservation option for leukemia patients as well (120). These techniques require testicular biopsies, which can negatively impact testicular growth, and inclusion in these experimental studies is reserved only for patients at very high risk of developing azoospermia.

### 5.1.3 Adverse events and late mortality after pediatric allo-HSCT (III)

In **Paper III**, almost all (85%) of the long-term survivors of pediatric allogeneic HSCT had a chronic health condition. With longer follow-up times, adverse events tended to increase both in numbers and severity in the TBI group. This can at least in part be explained by the more toxic procedures in the earliest era. Among the Bu-treated patients, the paradoxical increase in adverse events during the shortest follow-up times is most likely explained by the fact that more AML patients received Bu during the later era (instead of TBI), and the finding thus reflects the additive toxicity of previous leukemia treatments. The currently treated patients may experience less late toxicities from Bu as several improvements have been made in administering Bu that have decreased Bu-related morbidities and mortality (121). For example, the use of therapeutic dose-monitoring of Bu that allows more controlled systemic exposures was not available in the Nordic countries prior to 2000 but is currently a routine.

The conditioning regimen had a major impact on the burden of late effects. Non-hormonal chronic conditions were more frequent among patients conditioned with TBI or Bu-based regimens and in patients who had cGVHD. sTBI was shown to be the most toxic of the conditioning regimens and caused significantly more hormonal and non-hormonal late effects than fTBI. Hormonal late effects had somewhat higher prevalence among after TBI-based than Bu-based conditioning regimens.

sTBI has been abandoned, but fTBI is still widely used in pediatric ALL and associated with a higher burden of late adverse events compared to Bu. Our results support the notion that due to the high burden of late toxicities the use of TBI in children should be limited and, if possible, omitted. It is yet to be shown if TBI can be replaced in the treatment of childhood ALL without compromising survival. Cy-based conditioning regimens were clearly the least toxic, and they were used in SAA patients whose treatment prior to allo-HSCT did not include cytotoxic drugs.

Transplantation after relapse in CR2 did not associate with a significantly higher burden of late effects but there was a higher need of hormone replacement therapy in relapsed patients.

Among the late causes of death, extensive cGVHD was the major cause of late mortality. Two cardiovascular deaths more than 20 years after HSCT in relatively young patients are indicative that allo-HSCT survivors may experience serious cardiovascular events significantly earlier compared to the general population (42, 84). Our results confirm the need of lifelong follow-up after allo-HSCT in childhood.

#### 5.1.4 Long-term health-related outcomes after allo-HSCT for childhood AML (IV)

The overall survival after allo-HSCT and chemotherapy only is comparable in the latest studies (36, 37) and allo-HSCT is not recommended in first remission for pediatric AML in general due to the higher burden of side effects (37). In **Paper IV** we confirm the high burden of late effects associated with allo-HSCT reflected by the higher use of medications, higher prevalence of endocrinopathies, limitations in physical activities as well as more neurological disorders than among the AML survivors treated with chemotherapy only. Our results support sparing the low-risk patients from allo-HSCT and the toxicities associated with allo-HSCT. Although the currently treated childhood AML patients do not receive TBI, Bu is still widely used and was associated with a higher prevalence of cGVHD and significant morbidity in our study.

Half of the survivors had received TBI and half Bu. A few patients treated during the earliest era may also have been recipients of the more toxic sTBI. There was a strong association between TBI and cataracts, and although the highest prevalence of endocrine problems was seen after TBI, a significant proportion of survivors treated with Bu reported endocrinopathies. However, secondary malignancies were diagnosed only after TBI. Our follow-up may be too short for detecting secondary cancers after BU and cognitive impairments were not captured objectively.

From a clinical point of view, the choice is between allo-HSCT in CR1 or reserving allo-HSCT to CR2 for only those who relapse. Based on our results it seems that allo-HSCT in CR1 vs. CR2 does not make a large difference on the overall burden of late effects (**Papers III and IV**) but associates with an increased risk for cardiovascular disorders in AML survivors due to higher cumulative doses of anthracyclines. The higher prevalence of gastrointestinal disorders after relapse is most likely explained by the higher prevalence of cGVHD among the relapsed patients in our study. However, the influence of the higher cumulative doses of anthracyclines or alkylating agents on gastrointestinal disorders cannot be excluded (122). Considering that metabolic syndrome and insulin resistance are frequently described conditions following allo-HSCT, it is notable that none of the transplanted AML survivors in our study had been diagnosed with type 2 diabetes.

One of the most unfortunate consequences of the myeloablative conditioning regimens used for allo-HSCT is infertility. The several reported natural conceptions in this cohort indicate that some children can retain their fertility even after very intensive treatments followed by myeloablative allo-HSCT. In line with previous reports, a high proportion (4 out of the 12 reported pregnancies) ended in spontaneous miscarriages while the completed pregnancies resulted in healthy offspring (70, 123). The previously reported fertility rates among childhood AML patients treated according to the same AML protocols but without allo-HSCT did not differ from siblings (110) indicating that survivors treated according to the NOPHO-AML treatment protocols without allo-HSCT have a good chance of retaining their gonadal function and fertility. Therefore, one of the major advantages in sparing low risk

patients allo-HSCT would be the higher chance of preserved fertility when highly gonadotoxic conditioning with Bu is avoided. Less gonadotoxic alternatives to Bu are needed in the conditioning regimens of pediatric AML.

A future study comparing allo-HSCT and chemotherapy only could consider limiting the allo-HSCT cohort to the recipients of Bu-based conditioning regimens transplanted in CR1 for a fairer comparison. Considering the relatively high number of reported pregnancies, follow-up studies focusing on fertility issues and pregnancy outcomes in this cohort would also be interesting to conduct.

### 5.1.5 Strengths and limitations

The first three papers, **Papers I–III**, used detailed data collected from high-quality medical records. We were able to include 91% of the surviving cohort; only 9% was lost to follow-up and we could not retrieve their medical records. There is always a potential selection bias when not all eligible patients are included but due to the small number of missing patients it is unlikely that this potential bias would substantially alter our results. Although the data had been recorded prospectively the data collection for this study was performed retrospectively and some data was harder to obtain through the medical records. The data relies largely on the documentation done by the treating clinicians and could be influenced by the issues discussed during the outpatient visits. However, the follow-up programs included pre-defined screening and contacts with different specialists during the visits and were similar between the two transplant centers. Most patients met a pediatric endocrinologist for evaluations of pubertal development. Establishing exact figures for fertility/infertility through medical records can be difficult. Male survivors may be reluctant to leave sperm samples and establishing data on pregnancies in their partners is limited as it may not always be noted in medical records or paternity verified. In addition, the wish to become a parent or data on involuntary childlessness is seldom noted in the medical records. In **Paper III** we lacked more detailed objective neurocognitive follow-up data and Karnofsky scores. Recording of some milder neurocognitive deficits may have been missed at the annual visits and the neurocognitive late effects may be underestimated in this study considering that in **Paper IV**, almost one third of the transplanted patients reported neurological or neurocognitive dysfunction through self-report.

In **Paper IV**, data was collected from a population-based cohort and the participation rate was good, 65%. In addition, the survivors had been treated according to the same Nordic AML treatment protocols. However, the data relies on self-report on adverse outcomes and medications. It also relies on how well the physicians screened and informed the patients of their medical conditions. Possible local differences in the criteria for initiating medical treatment can influence the results comparing medications. However, considering that the data was collected from two well-investigated patient groups with regular follow-up visits the risk of information bias between these two AML treatment groups can be considered low. Although the data from the conventionally treated patients was collected four years earlier, the time from AML diagnosis was comparable between the two groups and might give a

fairer comparison between the two treatment modalities. It should be noted that although both cohorts can be considered young, the conventionally treated patients were significantly younger, and this may impact our results for outcomes that are strongly correlated with increasing age. In statistical analyses we try to control for this by adjusting for both the time from diagnosis and age at questionnaire when comparing the two AML treatment groups. The indications for allo-HSCT have changed over the years and the conditioning regimens have been altered in an attempt to minimize both immediate and late toxicities. During the earlier era patients were allocated to allo-HSCT in CR1 by biological chance whereas during the later era mainly high-risk patients have proceeded to allo-HSCT in CR1, and this can introduce confounding by indication among the more recently treated patients.

A higher proportion of patients transplanted in CR2 had cGVHD which might possibly interfere with the interpretation of the effect CR status at allo-HSCT has on the spectrum and severity of the late effects. However, we found very little difference in long-term outcomes in relation to CR status, similar to results in **Paper III**. In **Paper IV**, the higher prevalence of cGVHD in CR2 might have emphasized the burden of late effects after allo-HSCT in CR2, but our results showed mainly an increase in gastrointestinal disorders after allo-HSCT in CR2 in univariable comparisons within allo-HSCT.

In the sibling-pairs comparisons power was lost as only 53 allo-HSCT had a sibling comparison available. All the statisticians consulted advised against pooling of the allo-HSCT survivors' sibling data with the previously collected data from siblings of survivors treated with chemotherapy only. The sibling comparisons were done by using conditional regression where only the sibling pairs were compared. In sibling comparisons there is bound to be some information bias in medical conditions due to the fact that most siblings had had scarce contact with health care and had not been screened for medical conditions in the same manner as the AML survivors.

#### **5.1.6 Summary and conclusions**

Allo-HSCT has offered cure for numerous children and adolescents who would otherwise have been lost. However, our studies confirm the significant burden of late effects after allo-HSCT. Our results suggest that it may ultimately be the conditioning regimens and cGVHD that define the burden of late toxicities after allo-HSCT. Especially endocrine disorders showed strong correlation with the type of conditioning regimen used while non-hormonal chronic conditions had a strong correlation with cGVHD. We found significant morbidity after Bu-based conditioning regimens and most importantly, the gonadotoxic effects of Bu were comparable with TBI. The advantages achieved by the switch from TBI to Bu-based conditioning regimens includes somewhat lower frequency of endocrine disorders and less affected physical growth, and in males, there may be a higher chance of recovering spermatogenesis in the long-term. Also the risk for secondary malignancies is also most likely lower than after TBI but extended follow-up is needed to confirm this.

In the treatment of childhood myeloid leukemia, allo-HSCT after AML relapse can increase the risk of cardiovascular late toxicities. Allo-HSCT with Bu involves high risk of infertility which for many survivors is one of the most devastating late side effects after allo-HSCT. The presence of cGVHD is a major risk factor for a variety of late side effects and it also strongly correlates with compromised physical performance. Therefore, as long as allo-HSCT involves the risk of suffering from moderate or extensive cGVHD, proceeding to allo-HSCT in CR1 may significantly compromise health-related quality of life.

Although the risk of infertility is very high after allo-HSCT, the previously reported fertility rates of below 3% after allo-HSCT may be too pessimistic for childhood AML patients who receive allo-HSCT. We discovered a relatively large subpopulation of young women who had retained their fertility, and more pregnancies can be expected in more extended follow-up. However, the fertility window may be substantially shortened by the intensive treatments and further studies are warranted for evaluating the long-term gonadal function and for obtaining more comprehensive fertility rates.

Studies based on the current and previous treatments on the late effects after pediatric allo-HSCT have all come to the same conclusion: life-long surveillance, counseling and disease prevention is needed after this very intensive treatment. Reproduction issues need to be addressed in a timely fashion and they require good collaboration between the treating oncologists and reproductive specialists. The disease occurrence after HSCT does not follow the same patterns as in the general population and many diseases classically associated with older age can appear at an early age. Early recognition of the specific long-term toxicities after allo-HSCT is needed and can only be facilitated through increased awareness among the treating clinicians and primary health-care providers.

### **5.1.7 Future perspectives**

The future landscape of late effects after allo-HSCT will most likely be altered as treatments employ new approaches to minimize toxicities. Over time, the procedure of allo-HSCT can be expected to become more individualized, more effective and hopefully less toxic. Reducing Graft-versus-Host Disease while at the same time increasing Graft-versus-Leukemia would substantially improve the outcomes after pediatric allo-HSCT for leukemia. In order to achieve high-quality cure with allo-HSCT, less toxic conditioning regimens that do not compromise immediate survival are needed and the risk of extensive GVHD needs to be eliminated.

An improved understanding of the T-cell function and immune environment can help introduce more targeted novel immunotherapeutic approaches that could spare healthy tissues. Novel immunotherapies include engineering T-cells that mainly target tumor cells. Chimeric antigen receptor (CAR)-transgenic T cells provide an attractive approach for treating relapsed hematological malignancies. Other novel immunotherapy approaches include tumor-associated antigen vaccinations and monoclonal antibodies (124). In the treatment of AML, first results from a clinical trial employing the first antibody-drug



conjugate gemtuzumab ozogamicin (GO) in consolidation following RIC allo-HSCT indicate it to be well-tolerated with a 5-year overall survival of 61% (16) however possible late toxicities associated with GO are yet to be demonstrated.

Regardless of which new approaches become more established treatments, continued late effects research is needed to discover any possible long-term side effects of the new therapies. Also, the population of previously treated long-term survivors needs continued follow-up and repeated late effects studies for detecting any new, previously unknown long-term side effects.

## 6 ACKNOWLEDGEMENTS

I am deeply grateful to everyone who has helped me during the completion of this thesis.

First of all, I would like to thank my main supervisor **Kirsi Jahnukainen**, for if it weren't for her never-ending enthusiasm and expertise these projects might never have been completed.

I would also like to thank my co-supervisors: **Jacek Winiarski** for sharing his vast knowledge in the field of hematopoietic stem cell transplantation and for all the help and useful comments over the years. **Birgit Borgström** for her support and for sharing her expertise in the endocrine sequelae as well as fertility issues after allo-HSCT. **Henrik Hasle**, for contributing with his deep knowledge in childhood AML and for all his help with the AML projects.

My co-author and friend, **Anu Vatanen** for her peer support and friendship and company on conference trips. I would also like to express my gratitude to my other co-authors in Sweden and abroad, **Ulla M. Saarinen-Pihkala**, **Mervi Taskinen**, **Britt Gustafsson**, **Jonas Abrahamsson**, **Marianne Ifversen** and **Heidi Glosli** for their contribution to the projects as well as their constructive comments and help with the manuscripts.

Statistician **Björn Jonsson** in memoriam, our highly appreciated and efficient statistician who helped us tremendously with statistics in the first three papers and whose sudden death was a great loss. Statistician **Ulf Hammar**, for his patience with an amateur statistician and for all the help with statistical analyses and STATA.

I thank all **the participating survivors and their siblings**, as well as **all the treating hematologists and oncologists**, including (but not limited to) **Jacek Toporski**, **Johan Arvidson**, **Jón R Kristinsson**, **Päivi Lähteenmäki** and **Marianne Jarfelt**.

**All friends and colleagues at Astrid Lindgren Children's Hospital and everyone at the pediatric endocrinology unit** for creating a friendly working environment. **Olle Söder** for providing excellent research facilities and **Susanne Hallberg** for all her help over the years and even beyond retirement. **Jaana Vettenranta** for skillful technical assistance.

Special thanks to my research mentor and friend **Laura** for her support, all my friends from medical school including but not limited to **Inari**, **Jonna**, **Annukka**, **Saara**, **Pauliina**, **Eeva**, **Aida**, **Satu-Maria** and **Maijastiina** for their friendship and peer support in reaching this academic goal. All my friends in Sweden, Finland and abroad for being there for me.

**My mother Kaisu** for her unconditional love and support. My brother **Samppa** for setting an inspiring example of pursuing an academic career. My Swedish parents **Kajsa** and **Hasse** for all their help and support over the years. To my husband **Otto**, not only for his love and support that made this thesis possible but also for his exceptional technical skills in the field of computer sciences. Our three children **Harry**, **Milla** and **Ville** also deserve special thanks for forcing me to take breaks and for engaging me in other activities besides work, you mean the world to me world. Finally, I would like to dedicate this work to **my dear father, Harri**, in memoriam.

Generous financial support for completing this thesis was received from **the Stockholm County Council** (combined clinical residency and PhD training program), **the Swedish Childhood Cancer Foundation** and **Mary Bevés Childhood Cancer foundation**.

Sandra Götberg  
Anu Vatanen  
Christine Skwirut-Carlsson Jonas Abrahamsson  
Ulla M. Saarinen-Pihkala  
Heidi Glosli Lars Hagernäs Anders Tidblad  
Jacek Winiarski Sowmya Ramesh  
Aida Wahlgren Britt Masironi  
Jan-Bernd Stukenborg Pauliina Hartiala  
Farasat Zaman Karin Henning Laura Oehms Yunhan Zhao  
Svetlana Lajic Anna Ekesbo-Freisinger  
Andrea Merker Lena Wallensteen Satu-Maria Männistö  
Trausti Oskarsson Heidi Laitinen Nina Mogensen Lars Sävendahl  
Björn Jonsson Birgit Borgström Valentina Pampanini  
Saara Valkama Kirsi Jahnukainen Leif Karlsson  
Henrik Hasle Magdalena Kurek Therese Cedervall  
Eeva Haapio Martin Ritzén Valeria Messina Jonna Turunen  
Luise Landreh Inari Listo João Lopes Olle Söder Lena Sahlin  
Heini Kangasmäki  
Katja Sundström Bettina Celvin Konstantin Svechnikov Aida Kiviniemi  
Zelong Dou Iuulia Savchuk Halima Albalushi  
Ola Nilsson Laura Harjuoja-Madanat  
Eva Rylander Hedlund Annukka Vahtera Marie Lindefeldt  
Ulf Hammar Marianne Ifversen Andreas Pettersson  
Maijastiina Hietamäki  
Susanne Hallberg Mervi Taskinen  
Britt Gustafsson



## 7 REFERENCES

1. Rekers PE, Coulter MP, Warren SL. Effect of transplantation of bone marrow into irradiated animals. *Archives of Surgery*. 1950;60(4):635-67.
2. Thomas ED, Lochte HL, Jr., Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *The New England journal of medicine*. 1957;257(11):491-6.
3. Niederwieser D, Baldomero H, Szer J, Gratwohl M, Aljurf M, Atsuta Y, et al. Hematopoietic stem cell transplantation activity worldwide in 2012 and a SWOT analysis of the Worldwide Network for Blood and Marrow Transplantation Group including the global survey. *Bone marrow transplantation*. 2016;51(6):778-85.
4. Passweg JR, Baldomero H, Peters C, Gaspar HB, Cesaro S, Dreger P, et al. Hematopoietic SCT in Europe: data and trends in 2012 with special consideration of pediatric transplantation. *Bone marrow transplantation*. 2014;49(6):744-50.
5. Ballen KK, King RJ, Chitphakdithai P, Bolan CD, Jr., Agura E, Hartzman RJ, et al. The national marrow donor program 20 years of unrelated donor hematopoietic cell transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2008;14(9 Suppl):2-7.
6. Ivanovic Z. Hematopoietic stem cells in research and clinical applications: The “CD34 issue”. *World Journal of Stem Cells*. 2010;2(2):18-23.
7. Urbano-Ispizua A, Rozman C, Pimentel P, Solano C, de la Rubia J, Brunet S, et al. The number of donor CD3(+) cells is the most important factor for graft failure after allogeneic transplantation of CD34(+) selected cells from peripheral blood from HLA-identical siblings. *Blood*. 2001;97(2):383-7.
8. Servais S, Porcher R, Xhaard A, Robin M, Masson E, Larghero J, et al. Pre-transplant prognostic factors of long-term survival after allogeneic peripheral blood stem cell transplantation with matched related/unrelated donors. *Haematologica*. 2014;99(3):519-26.
9. Janeway CA Jr TP, Walport M, et al. *Immunobiology: The Immune System in Health and Disease* 2001.
10. Petersdorf EW. Genetics of graft-versus-host disease: The major histocompatibility complex. *Blood Reviews*. 2013;27(1):1-12.
11. Tiercy JM. How to select the best available related or unrelated donor of hematopoietic stem cells? *Haematologica*. 2016;101(6):680-7.
12. Tabilio A, Falzetti F, Zei T, De Ioanni M, Bonifacio E, Battelli F, et al. Graft engineering for allogeneic haploidentical stem cell transplantation. *Blood cells, molecules & diseases*. 2004;33(3):274-80.
13. Willasch AM, Peters C, Sedlacek P, Dalle J-H, Graphakos S, Yesilipek A, et al. Myeloablative Conditioning for First Allogeneic Hematopoietic Stem Cell Transplantation in Children with ALL: Total Body Irradiation or Chemotherapy? - a Multicenter EBMT-PDWP Study. *Blood*. 2017;130(Suppl 1):911-.
14. Atilla E. A Review of Myeloablative vs Reduced Intensity/Non-Myeloablative Regimens in Allogeneic Hematopoietic Stem Cell Transplantations. 2017;34(1):1-9.
15. Andersson BS, Thall PF, Madden T, Couriel D, Wang X, Tran HT, et al. Busulfan systemic exposure relative to regimen-related toxicity and acute graft-versus-host disease: defining a therapeutic window for i.v. BuCy2 in chronic myelogenous leukemia. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2002;8(9):477-85.
16. Zahler S, Bhatia M, Ricci A, Roy S, Morris E, Harrison L, et al. A Phase I Study of Reduced-Intensity Conditioning and Allogeneic Stem Cell Transplantation Followed by Dose Escalation of Targeted Consolidation Immunotherapy with Gemtuzumab Ozogamicin in Children and Adolescents with CD33+ Acute Myeloid Leukemia. *Biology of*

- blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2016;22(4):698-704.
17. Bitan M, He W, Zhang MJ, Abdel-Azim H, Ayas MF, Bielorai B, et al. Transplantation for children with acute myeloid leukemia: a comparison of outcomes with reduced intensity and myeloablative regimens. *Blood*. 2014;123(10):1615-20.
  18. Servais S, Baron F, Beguin Y. Allogeneic hematopoietic stem cell transplantation (HSCT) after reduced intensity conditioning. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis*. 2011;44(2):205-10.
  19. Satwani P, Morris E, Bradley MB, Bhatia M, van de Ven C, Cairo MS. Reduced intensity and non-myeloablative allogeneic stem cell transplantation in children and adolescents with malignant and non-malignant diseases. *Pediatric blood & cancer*. 2008;50(1):1-8.
  20. Beres AJ, Drobyski WR. The Role of Regulatory T Cells in the Biology of Graft Versus Host Disease. *Frontiers in immunology*. 2013;4.
  21. Hill L, Alousi A, Kebriaei P, Mehta R, Rezvani K, Shpall E. New and emerging therapies for acute and chronic graft versus host disease. *Therapeutic advances in hematology*. 2018;9(1):21-46.
  22. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2003;9(4):215-33.
  23. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2005;11(12):945-56.
  24. Flowers ME, Inamoto Y, Carpenter PA, Lee SJ, Kiem HP, Petersdorf EW, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. *Blood*. 2011;117(11):3214-9.
  25. Cho BS, Min CK, Eom KS, Kim YJ, Kim HJ, Lee S, et al. Feasibility of NIH consensus criteria for chronic graft-versus-host disease. *Leukemia*. 2009;23(1):78-84.
  26. Wagner JE, Kernan NA, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. *Lancet (London, England)*. 1995;346(8969):214-9.
  27. Bonini C, Peccatori J, Stanghellini MTL, Vago L, Bondanza A, Cieri N, et al. Haploidentical HSCT: a 15-year experience at San Raffaele. *Bone marrow transplantation*. 2015;50:S67.
  28. Ruutu T, van Biezen A, Hertenstein B, Henseler A, Garderet L, Passweg J, et al. Prophylaxis and treatment of GVHD after allogeneic haematopoietic SCT: a survey of centre strategies by the European Group for Blood and Marrow Transplantation. *Bone marrow transplantation*. 2012;47(11):1459-64.
  29. Klassen J. The role of photopheresis in the treatment of graft-versus-host disease. *Current Oncology*. 2010;17(2):55-8.
  30. Thomas ED, Buckner CD, Banaji M, Clift RA, Fefer A, Flournoy N, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Blood*. 1977;49(4):511-33.
  31. Bleakley M, Lau L, Shaw PJ, Kaufman A. Bone marrow transplantation for paediatric AML in first remission: a systematic review and meta-analysis. *Bone marrow transplantation*. 2002;29(10):843-52.
  32. Creutzig U, van den Heuvel-Eibrink MM, Gibson B, Dworzak MN, Adachi S, de Bont E, et al. Diagnosis and management of acute myeloid leukemia in children and

- adolescents: recommendations from an international expert panel. *Blood*. 2012;120(16):3187-205.
33. Creutzig U, Zimmermann M, Reinhardt D, Dworzak M, Sary J, Lehnbecher T. Early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis of the multicenter clinical trials AML-BFM 93 and AML-BFM 98. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2004;22(21):4384-93.
34. Molgaard-Hansen L, Mottonen M, Glosli H, Jonmundsson GK, Abrahamsson J, Hasle H. Early and treatment-related deaths in childhood acute myeloid leukaemia in the Nordic countries: 1984-2003. *British journal of haematology*. 2010;151(5):447-59.
35. Zwaan CM, Kolb EA, Reinhardt D, Abrahamsson J, Adachi S, Aplenc R, et al. Collaborative Efforts Driving Progress in Pediatric Acute Myeloid Leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2015;33(27):2949-62.
36. Hasle H. A critical review of which children with acute myeloid leukaemia need stem cell procedures. *British journal of haematology*. 2014;166(1):23-33.
37. Niewerth D, Creutzig U, Bierings MB, Kaspers GJ. A review on allogeneic stem cell transplantation for newly diagnosed pediatric acute myeloid leukemia. *Blood*. 2010;116(13):2205-14.
38. Abrahamsson J, Clausen N, Gustafsson G, Hovi L, Jonmundsson G, Zeller B, et al. Improved outcome after relapse in children with acute myeloid leukaemia. *British journal of haematology*. 2007;136(2):229-36.
39. Meshinchi S, Alonzo TA, Stirewalt DL, Zwaan M, Zimmerman M, Reinhardt D, et al. Clinical implications of FLT3 mutations in pediatric AML. *Blood*. 2006;108(12):3654-61.
40. Nordic Soc Pediat Hematology O. NOPHO-DBH AML 2012 Protocol 2013 [cited 2018. 2013-01-17:[Available from: [https://www.skion.nl/workspace/uploads/NOPHO-DBH-AML-2012-Protocol\\_v2-1\\_17012013.pdf](https://www.skion.nl/workspace/uploads/NOPHO-DBH-AML-2012-Protocol_v2-1_17012013.pdf)].
41. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorrow ML, Boeckh M, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. *The New England journal of medicine*. 2010;363(22):2091-101.
42. Bhatia S, Francisco L, Carter A, Sun CL, Baker KS, Gurney JG, et al. Late mortality after allogeneic hematopoietic cell transplantation and functional status of long-term survivors: report from the Bone Marrow Transplant Survivor Study. *Blood*. 2007;110(10):3784-92.
43. Bresters D, van Gils IC, Kollen WJ, Ball LM, Oostdijk W, van der Bom JG, et al. High burden of late effects after haematopoietic stem cell transplantation in childhood: a single-centre study. *Bone marrow transplantation*. 2010;45(1):79-85.
44. Baker KS, Bresters D, Sande JE. The burden of cure: long-term side effects following hematopoietic stem cell transplantation (HSCT) in children. *Pediatric clinics of North America*. 2010;57(1):323-42.
45. Bresters D, Lawitschka A, Cugno C, Pötschger U, Dalissier A, Michel G, et al. Incidence and severity of crucial late effects after allogeneic HSCT for malignancy under the age of 3 years: TBI is what really matters. *Bone marrow transplantation*. 2016;51(11):1482-9.
46. Armenian SH, Sun CL, Kawashima T, Arora M, Leisenring W, Sklar CA, et al. Long-term health-related outcomes in survivors of childhood cancer treated with HSCT versus conventional therapy: a report from the Bone Marrow Transplant Survivor Study (BMTSS) and Childhood Cancer Survivor Study (CCSS). *Blood*. 2011;118(5):1413-20.
47. Carreras E. How I manage sinusoidal obstruction syndrome after haematopoietic cell transplantation. *British journal of haematology*. 2015;168(4):481-91.

48. Khosla J, Yeh AC, Spitzer TR, Dey BR. Hematopoietic stem cell transplant-associated thrombotic microangiopathy: current paradigm and novel therapies. *Bone marrow transplantation*. 2017.
49. Hassan Z. Management of refractory hemorrhagic cystitis following hematopoietic stem cell transplantation in children. *Pediatric transplantation*. 2011;15(4):348-61.
50. Cutler C, Li S, Kim HT, Laglenne P, Szeto KC, Hoffmeister L, et al. Mucositis after Allogeneic Hematopoietic Stem Cell Transplantation: A Cohort Study of Methotrexate- and Non-Methotrexate-Containing Graft-versus-Host Disease Prophylaxis Regimens. *Biology of Blood and Marrow Transplantation*. 2005;11(5):383-8.
51. Olsson R, Remberger M, Schaffer M, Berggren DM, Svahn BM, Mattsson J, et al. Graft failure in the modern era of allogeneic hematopoietic SCT. *Bone marrow transplantation*. 2013;48(4):537-43.
52. Baron F, Maris MB, Sandmaier BM, Storer BE, Sorror M, Diaconescu R, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2005;23(9):1993-2003.
53. Ferra C, Sanz J, Diaz-Perez MA, Morgades M, Gayoso J, Cabrera JR, et al. Outcome of graft failure after allogeneic stem cell transplant: study of 89 patients. *Leukemia & lymphoma*. 2015;56(3):656-62.
54. Jacobsohn DA, Vogelsang GB. Acute graft versus host disease. *Orphanet Journal of Rare Diseases*. 2007;2:35.
55. Cohen A, Vanlint MT, Lavagetto A, Chiodi S, Spinelli S, Bacigalupo A, et al. Pubertal development and fertility in children after bonemarrow transplantation. *Bone marrow transplantation*. 1991;8:16-20.
56. Sanders JE. Endocrine complications of high-dose therapy with stem cell transplantation. *Pediatric transplantation*. 2004;8:39-50.
57. Cohen A, Békássy AN, Gaiero A, Faraci M, Zecca S, Tichelli A, et al. Endocrinological late complications after hematopoietic SCT in children. *Bone marrow transplantation*. 2008;41(SUPPL. 2):S43-S8.
58. Bresters D, Lawitschka A, Cugno C, Potschger U, Dalissier A, Michel G, et al. Incidence and severity of crucial late effects after allogeneic HSCT for malignancy under the age of 3 years: TBI is what really matters. *Bone marrow transplantation*. 2016;51(11):1482-9.
59. Pulsipher MA, Skinner R, McDonald GB, Hingorani S, Armenian SH, Cooke KR, et al. National Cancer Institute, National Heart, Lung and Blood Institute/Pediatric Blood and Marrow Transplantation Consortium First International Consensus Conference on late effects after pediatric hematopoietic cell transplantation: the need for pediatric-specific long-term follow-up guidelines. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2012;18(3):334-47.
60. Sanders JE, Hoffmeister PA, Woolfrey AE, Carpenter PA, Storer BE, Storb RF, et al. Thyroid function following hematopoietic cell transplantation in children: 30 years' experience. *Blood*. 2009;113(2):306-8.
61. Vrooman LM, Millard HR, Brazauskas R, Majhail NS, Battiwalla M, Flowers ME, et al. Survival and Late Effects after Allogeneic Hematopoietic Cell Transplantation for Hematologic Malignancy at Less than Three Years of Age. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2017;23(8):1327-34.
62. Shalitin S, Pertman L, Yackobovitch-Gavan M, Yaniv I, Lebenthal Y, Phillip M, et al. Endocrine and Metabolic Disturbances in Survivors of Hematopoietic Stem Cell Transplantation in Childhood and Adolescence. *Hormone research in paediatrics*. 2018.



63. Borgmann-Staudt A, Rendtorff R, Reinmuth S, Hohmann C, Keil T, Schuster FR, et al. Fertility after allogeneic haematopoietic stem cell transplantation in childhood and adolescence. *Bone marrow transplantation*. 2012;47(2):271-6.
64. Mertens AC, Ramsay NK, Kouris S, Neglia JP. Patterns of gonadal dysfunction following bone marrow transplantation. *Bone marrow transplantation*. 1998;22(4):345-50.
65. Miyoshi Y, Yasuda K, Miyamura T, Miyashita E, Hashii Y, Ozono K. Anti-müllerian hormone is a useful marker of gonadotoxicity in girls treated for cancer: A prospective study. *Hormone research in paediatrics*. 2015;84:479.
66. De Vos M, Devroey P, Fauser BCJM. Primary ovarian insufficiency. *The Lancet*. 376(9744):911-21.
67. Herrmann T. [Radiation reactions in the gonads: importance in patient counseling]. *Strahlentherapie und Onkologie : Organ der Deutschen Röntgengesellschaft [et al]*. 1997;173(10):493-501.
68. Bakker B, Massa GG, Oostdijk W, Van Weel-Sipman MH, Vossen JM, Wit JM. Pubertal development and growth after total-body irradiation and bone marrow transplantation for haematological malignancies. *European journal of pediatrics*. 2000;159(1-2):31-7.
69. Howell SJ, Radford JA, Ryder WDJ, Shalet SM. Testicular Function After Cytotoxic Chemotherapy: Evidence of Leydig Cell Insufficiency. *Journal of Clinical Oncology*. 1999;17(5):1493-.
70. Salooja N, Szydlo RM, Socie G, Rio B, Chatterjee R, Ljungman P, et al. Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey. *Lancet (London, England)*. 2001;358(9278):271-6.
71. Loren AW, Chow E, Jacobsohn DA, Gilleece M, Halter J, Joshi S, et al. Pregnancy After Hematopoietic-cell Transplantation: A Report From the Late Effects Working Committee of the Center for International Blood and Marrow Transplant Research (CIBMTR). *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2011;17(2):157-66.
72. Teh WT, Stern C, Chander S, Hickey M. The Impact of Uterine Radiation on Subsequent Fertility and Pregnancy Outcomes. *BioMed Research International*. 2014;2014.
73. Rovo A, Aljurf M, Chiodi S, Spinelli S, Salooja N, Sucak G, et al. Ongoing graft-versus-host disease is a risk factor for azoospermia after allogeneic hematopoietic stem cell transplantation: a survey of the Late Effects Working Party of the European Group for Blood and Marrow Transplantation. *Haematologica*. 2013;98(3):339-45.
74. Kinsella TJ. Effects of radiation therapy and chemotherapy on testicular function. *Progress in clinical and biological research*. 1989;302:157-71; discussion 72-7.
75. Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, et al. Fertility of Male Survivors of Childhood Cancer: A Report From the Childhood Cancer Survivor Study. *Journal of Clinical Oncology*. 2010;28(2):332-9.
76. Rovo A, Tichelli A, Passweg JR, Heim D, Meyer-Monard S, Holzgreve W, et al. Spermatogenesis in long-term survivors after allogeneic hematopoietic stem cell transplantation is associated with age, time interval since transplantation, and apparently absence of chronic GvHD. *Blood*. 2006;108(3):1100-5.
77. Winther JF, Boice JD, Jr., Frederiksen K, Bautz A, Mulvihill JJ, Stovall M, et al. Radiotherapy for childhood cancer and risk for congenital malformations in offspring: a population-based cohort study. *Clinical genetics*. 2009;75(1):50-6.
78. Madanat-Harjuoja LM, Malila N, Lahteenmaki P, Pukkala E, Mulvihill JJ, Boice JD, Jr., et al. Risk of cancer among children of cancer patients - a nationwide study in Finland. *International journal of cancer*. 2010;126(5):1196-205.
79. Leiper AD, Stanhope R, Lau T, Grant DB, Blacklock H, Chessells JM, et al. The effect of total body irradiation and bone marrow transplantation during childhood and

- adolescence on growth and endocrine function. *British journal of haematology*. 1987;67(4):419-26.
80. Mostoufi-Moab S, Ginsberg JP, Bunin N, Zemel B, Shults J, Leonard MB. Bone density and structure in long-term survivors of pediatric allogeneic hematopoietic stem cell transplantation. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2012;27(4):760-9.
  81. Ranke MB, Schwarze CP, Dopfer R, Klingebiel T, Scheel-Walter HG, Lang P, et al. Late effects after stem cell transplantation (SCT) in children - growth and hormones. *Bone marrow transplantation*. 2005;35:S77-S81.
  82. Sanders JE, Guthrie KA, Hoffmeister PA, Woolfrey AE, Carpenter PA, Appelbaum FR. Final adult height of patients who received hematopoietic cell transplantation in childhood. *Blood*. 2005;105(3):1348-54.
  83. Swerdlow AJ, Cooke R, Beckers D, Borgstrom B, Butler G, Carel JC, et al. Cancer Risks in Patients Treated With Growth Hormone in Childhood: The SAGhE European Cohort Study. *The Journal of clinical endocrinology and metabolism*. 2017;102(5):1661-72.
  84. Chow EJ, Mueller BA, Baker KS, Cushing-Haugen KL, Flowers ME, Martin PJ, et al. Cardiovascular hospitalizations and mortality among recipients of hematopoietic stem cell transplantation. *Annals of internal medicine*. 2011;155(1):21-32.
  85. Rovó A, Tichelli A. Cardiovascular Complications in Long-Term Survivors After Allogeneic Hematopoietic Stem Cell Transplantation. *Seminars in Hematology*. 2012;49(1):25-34.
  86. Chow EJ, Baker KS, Lee SJ, Flowers ME, Cushing-Haugen KL, Inamoto Y, et al. Influence of conventional cardiovascular risk factors and lifestyle characteristics on cardiovascular disease after hematopoietic cell transplantation. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2014;32(3):191-8.
  87. Oudin C, Simeoni MC, Sirvent N, Contet A, Begu-Le Coroller A, Bordigoni P, et al. Prevalence and risk factors of the metabolic syndrome in adult survivors of childhood leukemia. *Blood*. 2011;117(17):4442-8.
  88. Socie G, Baker KS, Bhatia S. Subsequent malignant neoplasms after hematopoietic cell transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2012;18(1 Suppl):S139-50.
  89. Vatanen A, Sarkola T, Ojala TH, Turanlahti M, Jahnukainen T, Saarinen-Pihkala UM, et al. Radiotherapy-related arterial intima thickening and plaque formation in childhood cancer survivors detected with very-high resolution ultrasound during young adulthood. *Pediatric blood & cancer*. 2015;62(11):2000-6.
  90. Mulrooney DA, Yeazel MW, Kawashima T, Mertens AC, Mitby P, Stovall M, et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. *BMJ (Clinical research ed)*. 2009;339:b4606.
  91. Nakaseko C, Ozawa S, Sakaida E, Sakai M, Kanda Y, Oshima K, et al. Incidence, risk factors and outcomes of bronchiolitis obliterans after allogeneic stem cell transplantation. *Int J Hematol*. 2011;93(3):375-82.
  92. Nagasawa M, Mitsuiki N, Aoki Y, Ono T, Isoda T, Imai K, et al. Effect of reduced-intensity conditioning and the risk of late-onset non-infectious pulmonary complications in pediatric patients. *European journal of haematology*. 2017;99(6):525-31.
  93. Bonnesen TG, Winther JF, Andersen KK, Asdahl PH, de Fine Licht S, Gudmundsdottir T, et al. Liver diseases in Adult Life after Childhood Cancer in Scandinavia (ALiCCS): A population-based cohort study of 32,839 one-year survivors. *International journal of cancer*. 2018;142(4):702-8.

94. Socie G, Salooja N, Cohen A, Rovelli A, Carreras E, Locasciulli A, et al. Nonmalignant late effects after allogeneic stem cell transplantation. *Blood*. 2003;101(9):3373-85.
95. Cohen EP. Chronic kidney disease after hematopoietic stem cell transplantation. 2010;30(6):627-34.
96. Baker KS, DeFor TE, Burns LJ, Ramsay NK, Neglia JP, Robison LL. New malignancies after blood or marrow stem-cell transplantation in children and adults: incidence and risk factors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2003;21(7):1352-8.
97. Mulcahy Levy JM, Tello T, Giller R, Wilkening G, Quinones R, Keating AK, et al. Late effects of total body irradiation and hematopoietic stem cell transplant in children under 3 years of age. *Pediatric blood & cancer*. 2013;60(4):700-4.
98. Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011;29(5):551-65.
99. Ness KK, Bhatia S, Baker K, et al. Performance limitations and participation restrictions among childhood cancer survivors treated with hematopoietic stem cell transplantation: The bone marrow transplant survivor study. *Archives of Pediatrics & Adolescent Medicine*. 2005;159(8):706-13.
100. Tichelli A, Labopin M, Rovó A, Badoglio M, Arat M, van Lint MT, et al. Increase of Suicide and Accidental Death After Hematopoietic Stem Cell Transplantation: A Cohort Study on Behalf of the Late Effects Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Cancer*. 2013;119(11):2012-21.
101. Barlogis V, Auquier P, Bertrand Y, Chastagner P, Plantaz D, Poiree M, et al. Late cardiomyopathy in childhood acute myeloid leukemia survivors: a study from the L.E.A. program. *Haematologica*. 2015;100(5):e186-9.
102. Schultz KAP, Chen L, Chen Z, Kawashima T, Oeffinger KC, Woods WG, et al. Health conditions and quality of life in survivors of childhood acute myeloid leukemia comparing post remission chemotherapy to BMT: A report from the children's oncology group. *Pediatric blood & cancer*. 2014;61(4):729-36.
103. Blijdorp K, van Waas M, van der Lely A-J, Pieters R, van den Heuvel-Eibrink M, Neggers S. Endocrine sequelae and metabolic syndrome in adult long-term survivors of childhood acute myeloid leukemia. *Leukemia Research*. 2013;37(4):367-71.
104. Klusmann JH, Reinhardt D, Zimmermann M, Kremens B, Vormoor J, Dworzak M, et al. The role of matched sibling donor allogeneic stem cell transplantation in pediatric high-risk acute myeloid leukemia: results from the AML-BFM 98 study. *Haematologica*. 2012;97(1):21-9.
105. Leung W, Hudson MM, Strickland DK, Phipps S, Srivastava DK, Ribeiro RC, et al. Late Effects of Treatment in Survivors of Childhood Acute Myeloid Leukemia. *Journal of Clinical Oncology*. 2000;18(18):3273-9.
106. Leahey AM, Teunissen H, Friedman DL, Moshang T, Lange BJ, Meadows AT. Late effects of chemotherapy compared to bone marrow transplantation in the treatment of pediatric acute myeloid leukemia and myelodysplasia. *Medical and pediatric oncology*. 1999;32(3):163-9.
107. Liesner RJ, Leiper AD, Hann IM, Chessells JM. Late effects of intensive treatment for acute myeloid leukemia and myelodysplasia in childhood. *Journal of Clinical Oncology*. 1994;12(5):916-24.
108. Mulrooney DA, Dover DC, Li S, Yasui Y, Ness KK, Mertens AC, et al. Twenty years of follow-up among survivors of childhood and young adult acute myeloid leukemia: a report from the Childhood Cancer Survivor Study. *Cancer*. 2008;112(9):2071-9.
109. Molgaard-Hansen L, Glosli H, Jahnukainen K, Jarfelt M, Jonmundsson GK, Malmros-Svennilson J, et al. Quality of health in survivors of childhood acute myeloid

- leukemia treated with chemotherapy only: a NOPHO-AML study. *Pediatric blood & cancer*. 2011;57(7):1222-9.
110. Molgaard-Hansen L, Skou AS, Juul A, Glosli H, Jahnukainen K, Jarfelt M, et al. Pubertal Development and Fertility in Survivors of Childhood Acute Myeloid Leukemia Treated With Chemotherapy Only: A NOPHO-AML Study. *Pediatric blood & cancer*. 2013;60(12):1988-95.
  111. Skou AS, Glosli H, Jahnukainen K, Jarfelt M, Jonmundsson GK, Malmros-Svennilson J, et al. Renal, gastrointestinal, and hepatic late effects in survivors of childhood acute myeloid leukemia treated with chemotherapy only--a NOPHO-AML study. *Pediatric blood & cancer*. 2014;61(9):1638-43.
  112. Jarfelt M, Andersen NH, Glosli H, Jahnukainen K, Jonmundsson GK, Malmros J, et al. Cardiac function in survivors of childhood acute myeloid leukemia treated with chemotherapy only: a NOPHO-AML study. *European journal of haematology*. 2016;97(1):55-62.
  113. Robison LL, Mertens AC, Boice JD, Breslow NE, Donaldson SS, Green DM, et al. Study design and cohort characteristics of the Childhood Cancer Survivor Study: a multi-institutional collaborative project. *Medical and pediatric oncology*. 2002;38(4):229-39.
  114. Sanders JE, Woolfrey AE, Carpenter PA, Storer BE, Hoffmeister PA, Deeg HJ, et al. Late effects among pediatric patients followed for nearly 4 decades after transplantation for severe aplastic anemia. *Blood*. 2011;118(5):1421-8.
  115. Dolmans M-M, Marinescu C, Saussoy P, Van Langendonck A, Amorim C, Donnez J. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. *Blood*. 2010;116(16):2908-14.
  116. Dolmans M-M, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. *Fertility and sterility*. 2013;99(6):1514-22.
  117. Kelsey TW, McConville L, Edgar AB, Ungurianu AI, Mitchell RT, Anderson RA, et al. Follicle Stimulating Hormone is an accurate predictor of azoospermia in childhood cancer survivors. *PloS one*. 2017;12(7):e0181377.
  118. Condorelli R, Calogero AE, La Vignera S. Relationship between Testicular Volume and Conventional or Nonconventional Sperm Parameters. *International Journal of Endocrinology*. 2013;2013.
  119. Palermo GD, O'Neill CL, Chow S, Cheung S, Parrella A, Pereira N, et al. Intracytoplasmic sperm injection: state of the art in humans. *Reproduction (Cambridge, England)*. 2017;154(6):F93-f110.
  120. Jahnukainen K, Stukenborg JB. Present and Future Prospects of Male Fertility Preservation for Children and Adolescents. *Journal of Clinical Endocrinology & Metabolism*. 2012;97(12):4341-51.
  121. Ciurea SO, Andersson BS. Busulfan in Hematopoietic Stem Cell Transplantation. *Biology of Blood and Marrow Transplantation*. 2009;15(5):523-36.
  122. Goldsby R, Chen Y, Raber S, Li L, Diefenbach K, Shnorhavorian M, et al. Survivors of Childhood Cancer Have Increased Risk for Gastrointestinal Complications Later in Life. *Gastroenterology*. 2011;140(5):1464-71.e1.
  123. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. *Blood*. 1996;87(7):3045-52.
  124. Audehm S, Krackhardt AM. Specific Adoptive Cellular Immunotherapy in Allogeneic Stem Cell Transplantation. *Oncology research and treatment*. 2017;40(11):691-6.